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Manpower needs are not so easily estimated

IN A DOCUMENT put about by the Manpower Services Commission of the Department of Employment, the claim is made, amongst others, that in the off-shore oil industry 'immediate and continuing shortages of geologists, petroleum engineers, drillers and divers have been established'. The circumstances surrounding the report—*The discovery of off-shore oil and gas: manpower implications*—are such there must be the gravest doubts that it merits the extensive publicity it has so far received.

The commission, about which you will learn nothing at all on any page (or the cover) of the report—not even its address, telephone number or where it fits into ministerial structure, sponsored Mr David Begg of Nuffield College, Oxford to write a report which could be an overview of manpower implications of the discovery and exploitation of off-shore gas and oil. Mr Begg took only a couple of months over the draft and has now left to do postgraduate research in the United States. Subsequently Mr John Fyfe, of the commission's secretariat, 'up-dated, revised and expanded' the draft, but in publishing it (at least I suppose it's published although it has 'confidential' on the front page) the commission made the odd statement that the views were those of the authors and not necessarily those of the commission. It is mystifying to know why a report is being disseminated for 'interest and assistance' if the commission cannot stand behind it.

Mr Begg seems to have consulted and drawn extensively from about ten more specialised studies in his overview; it is unclear, however, how many people in the industry he spoke to. The result, as one critic put it, is an A+ for the college essay prize but no marks for producing a document of any help to policymakers in deciding what, if anything, should be done by government, industry and educational authorities.

The authors draw heavily on figures from the Petroleum Industry Training Board (PITB), also a Department of Employment agency. PITB forecasts for oil-related employment are based on a projected number of mobile drilling rigs which, the authors admit, was demonstrably in error as early as June 1974. These errors may partly spring from the nature of a multinational, ever-changing industry in which are all sorts of service companies which do not report operations and employment figures to the board. To go on to accept PITB projections on employment merely with the comment that they should be

treated as conservative estimates is a poor substitute for the detailed investigative work that is needed.

As it is, the report, echoing the PITB, sees the number of jobs for geologists/geophysicists rising from 440 in 1973 to 520 (misprinted as 570) in 1980; for petroleum from 190 to 330 in the same period, and for divers from 300 in 1974 to 650 in the late 1970s.

Can skilled men be provided in sufficient numbers? The report, supposedly aimed at this question, never really answers it at all. Amongst geologists, for instance, what evidence is there that there is a shortfall on the 200 that the PITB say can be employed at present? Or that there will be a shortfall on the 220 that could be used in 1980? None at all, and the authors never address the problem, instead tut-tutting that university geology courses are unsuited to the demands of petroleum geology. If the authors instead of rushing into print had chosen to talk to some of the major oil companies, they would have found that the companies run extensive training courses and prefer the unpre doctrinated graduate. They would also have found that the companies are acquiring geology graduates without difficulty in what one executive described as a buyer's market. And yet the report speaks of having 'established' an immediate and continuing shortage of geologists.

A similarly critical analysis could probably be applied in the other professions. There seems to be an all-too-ready desire to call any differential between present and future needs a shortfall without a serious examination of ways in which the oil industry is likely to respond. And since the industry is multinational, professionals that can't be found in Britain will probably be imported.

In the case of deep-sea diving, where skills will undoubtedly be in demand for many years, the authors choose to use the emotive turn of phrase rather than careful study. Perhaps 'inadequate training and a high fatality rate' is a correct summing-up of the scene, but what confidence can one have that this undocumented charge is anything more than much else in the report—hunch and hearsay?

It may well be true that there will be shortages of skills in the North Sea, but it would be dangerous to base any plan of action on the evidence of this report. The ability of the British government to monitor what is happening under its own nose must be brought into the most serious doubt by this document. □



THE newly formed United Kingdom section of the International Solar Energy Society has set itself a formidable task in the first year of its existence. It is preparing on its own initiative an assessment of the potential usefulness of solar energy in Britain, along the lines of reports recently prepared in Australia and the United States.

A working party of some forty experts in the various branches of solar energy use has been set up with the help of a grant of £5,000 from the Wolfson Foundation under its programme to support research into the better use of Britain's natural resources, and the report on solar energy should be published in September 1975.

Although the outlook for solar energy in the gloomy days of a British winter seems on the face of it distinctly bleak, a measure of the interest in its possibilities, even in northern latitudes, is given by the rapid growth of the UK section since its formation early in 1974. It now boasts a membership of more than 450, second only to the North American section.

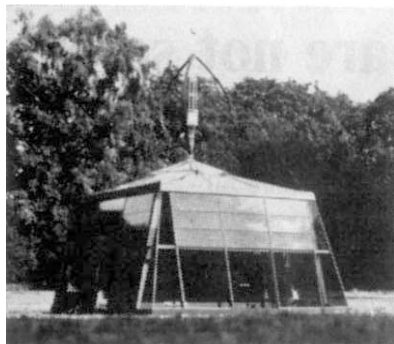
A definite check to research into the applications of solar energy in Britain was given earlier this year by the Central Policy Review Staff, the Think Tank, which recommended in its report on energy conservation that it would not be useful to increase government support for such research. One of the aims of the Solar Energy Society's report will undoubtedly be to try to persuade the government otherwise. By definition the members of the society believe that solar energy has a future in Britain in the short term, in the long term as a source of inexhaustible energy when fossil fuels are exhausted, and as one alternative to the large scale use of nuclear power. The problem as they see it is to pinpoint the areas of technology and basic research which can be most usefully developed to serve the special needs of a highly industrialised, densely populated country like Britain, with a climate which is far from ideal.

But there are ways in which solar energy could make an increased contribution to Britain's energy needs fairly soon, in providing supplementary water and space heating. The technology is available and there is already a small commercial solar heater industry in Britain. With the costs of gas and electricity all set to rise to 'realistic' levels, which will mean an eventual rise of some 30% in electricity prices, supplementary solar power, plumbed into existing hot water systems and suitably insulated, for water heating could become very attractive if it were relatively cheap and easy to install. One of the problems the report will have to consider in this context, how-

ever, is the feasibility of installing this sort of heater in existing buildings.

With regard to the longer term ideal of designing new houses and institutional buildings to take more account of solar radiation and perhaps incorporate solar heating devices, economics and social acceptability will probably

Solar energy in Britain



Solar collectors for heating

The UK Section of the Solar Energy Society has got off the ground with a bang. Eleanor Lawrence has been talking to some of its members about the report on the potential of solar energy for Britain now in preparation.

be the main barriers, although there are still technical problems to be evaluated and overcome.

Many of the other applications of solar energy to be considered in the report are far more speculative. Solar energy research is nothing if not multidisciplinary, and architects and engineers rub shoulders with plant breeders and photobiologists. Green plants have solved not only the problem of capturing sunlight but also the vexed question of how to store the energy derived from it, a problem which is currently exercising the brains of everybody involved in the utilisation of intermittent and irregular sources of power.

The section of the working party dealing with agricultural and biological systems will consider plants both as a source of materials, including food, and as a source of fuel. Research is already in progress in Britain to breed and select plants which make a better use of the available solar energy through a longer growing season and an extended range of growing conditions. As to fuel, the fossil kinds represent a non-renewable source of solar energy stored by plants. So, the argument goes, why not use

plants as a renewable source of fuel by converting plants and plant wastes to liquid fuels, gas and char by pyrolysis and fermentation. Problems the report will have to consider here are the availability of suitable plants, land and the technology needed for making use of the products. Plants grown for fuel may not necessarily have to compete for valuable land with plants grown for food, and it is here that research on the better use of available crops and the introduction of previously unconsidered types as food and/or fuel will be of prime importance. One of the main difficulties with any increase in the growing and harvesting of plants for whatever purpose will be the increased amounts of fertilisers needed. A major limiting factor is expected to be the availability of phosphate, a non-renewable resource produced by only a few countries. Recycling of phosphate and other non-renewable fertilisers is therefore an essential prerequisite before any such schemes can be put into operation. In the report prepared by the Australian Academy of Sciences, the long term nature of such work and the necessity for an early start on research was emphasised, a plea echoed by Professor David Hall, of the Department of Plant Science at King's College London, Chairman of the Agriculture and Biological Systems Section of the working party. If funds for such research are not made available very soon so that the problems and potentials can be properly identified, any useful contribution will take even longer to materialise than the twenty years envisaged by Professor Hall.

Similar considerations apply to work on the photochemical and photobiological conversion of solar energy. The study of the photosynthetic apparatus with the eventual aim of developing synthetic or semi-synthetic systems for using solar energy to split water to produce hydrogen in large amounts, say, has enormous long term implications but is threatened in the present times of financial stringency.

There is a greater industrial interest in photovoltaic solar cells because experience has been built up as a consequence of the space programme, but present terrestrial applications are limited to a few specialised uses as at present costs are very high. If components suitable for mass production could be developed costs would soon fall to economic levels opening up new possibilities for the generation of electric power.

Even if it eventually transpires that the potential for solar energy is indeed fairly limited in Britain—the Society's committee remains open-minded on this issue—Britain can hardly afford to miss out on the potentially valuable export market in solar technology. □

international news

EIGHT THOUSAND million francs in the French budget for scientific research means a growth of 13% over 1974—barely enough to keep pace with inflation. It has been described as a marking-time budget, and hardly more could have been expected in the present economic climate. Moreover the new government, coming after a long period when no decisions were taken, has not yet put forward a definitive policy on all aspects of science. Bernard Gregory, director of the Centre National de la Recherche Scientifique (CNRS), recently summed it up in an interview in *Le Monde*: "it won't do much harm to future prospects as the money has been distributed with a view to keeping open all options . . . but it is not a situation which can go on for many years".

The *enveloppe recherche*, as the budget is called, is only concerned with interministerial projects (which fall within the province of the Délégation Générale à la Recherche Scientifique et Technique (DGRST)). Military, telecommunication and aeronautics expenditures are the concern of their own ministries (in 1973 they came to 6,230 million francs). In 1967 2.2% of GNP was devoted to all research, now it is 1.7%. The increase in public expenditure between 1969 and 1973 was only 33%, compared with 69% in Italy and

Changes due in French science

from the staff of La Recherche

95% in Germany.

There will be very few new jobs; this is of particular concern to government laboratories for which the budget gives authority to take on permanently people of specific skills. In 1971, 432 research jobs were created, in 1973, 200; for 1975 only 156 are projected. The Minister of Industry and Research M d'Ornano, criticised for this running down of manpower, replied that one should add to this figure 194 technicians and 264 *vacataire* posts (research workers on short term contracts).

A controlled budget means a restriction on non-current expenditure for new projects. The public sector has not suffered too much in this way—construction of new laboratories or purchase of new equipment can be postponed—but private laboratories living on state funds will feel the pinch; many scientists could find themselves out of work. This was already happening in mid-1974 in the social sciences sector.

There are priority areas, mainly in

the fields of production and distribution of energy. After several stagnant years, the atomic energy commission starts to grow again by 26.7% in current francs. Most of this will go to reactor safety, treating of fuel elements and the storage of nuclear waste. The rest of the energy expenditure (up to F178.4 millions from F116.1 millions in 1974) will go mainly on thermonuclear fusion research and to a lesser degree on energy conservation, solar energy (F12 million) and geothermal energy (F5 million). Nobody contests the high priority for energy; even so, some deputies have qualms about the overwhelming and unthinking emphasis on electricity production.

Other priorities are biology and certain parts of the social sciences. Research with socio-economic ends (environment, urbanism, transport, pollution) still grows but less than it did in previous years. After much hesitation the launcher *Ariane* is kept thanks to deft budgetary transfers and since *Ariane* is interlocked with other projects, space is relatively well supported financially.

Fundamental research, however, as practised at CNRS and in the universities stagnates; there will only be a 7% growth in current francs, and this is causing much worry.

IN France, as in most western countries, the role of scientists in determining the major issues of science policy has been declining since the late 1960s. The large research institutions are, it is true, in general directed by scientists, but these people are so deeply involved in management that they act more as scientific administrators than as practising scientists. The 1958 reforms in these institutions led to the creation of two key bodies, the Délégation Générale à la Recherche Scientifique et Technique (DGRST), having an interministerial coordinating role in science policy under the Prime Minister and the Comité Consultatif de la Recherche Scientifique et Technique charged with advising the government on matters of research policy. This latter consists of a dozen members, *les douze sages* nominated by the government for periods of four years. *Les douze sages* was a lively and important body to start with. In particular, it prepared the research budget in liaison with the DGRST, and although its advice was not always followed it had an active

Wise but powerless

from the staff of La Recherche

role in the first years of the Fifth Republic, a time when many research bodies were constituted or reformed.

French science policy changed after 1965 under M Pompidou. The priority that research had possessed early in the Fifth Republic was no longer there, and ministers for research after 1965 had less political clout than their predecessors. The influence of *les douze sages* waned and that of administrators grew accordingly.

At present the committee has practically no influence and now that the government has announced a reform of science policy machinery, and particularly of the coordinating body of the DGRST, the role of the committee has come into question. The Minister for Industry and Research, M d'Ornano, said in a recent debate on the research budget in the National Assembly "maybe it is time to start those who are involved in research policy thinking about a problem which

has so far passed them by but to which they should not be indifferent: that of society's control over technological development . . . One of the first things we could do is bring non-scientists, elected politicians and consumer representatives into closer touch with the implementation of new technologies and the definition of new programmes". The president of *les douze sages*, M Brams, has received the mandate to think about possible reform of the committee and to make proposals relevant to d'Ornano's plan. The problem is that of non-scientists being involved in science policy decisions and this issue had already been aired in Mitterand's presidential campaign, when the idea had been put forward of a high ranking consultative committee on science policy whose members would include non-scientists.

The big question is, if twelve becomes fifteen or twenty will the scientific community and even the whole nation be better represented and will the committee have more power and authority than at present?

NIH Director edged out

by Colin Norman, Washington

For the second time in two years, the Director of the National Institutes of Health (NIH) has been fired, apparently because of disagreements with other key administration officials over how biomedical research should be managed and funded in the United States.

Last month, Dr Robert S. Stone, who had been NIH Director since May 1973, was asked to resign by Dr Charles C. Edwards, the top health official in the Department of Health, Education and Welfare (HEW). The event immediately evoked a chorus of protests from several prominent biomedical scientists who charged in a statement put out by the Federation of American Scientists that the NIH is being subjected to "unwarranted and counterproductive political control".

Although no official reasons have yet been given, Stone's downfall is widely believed to have resulted from his opposition to further cutbacks in the NIH's budget and also from his failure to see eye-to-eye with Edwards on the role that the NIH should play in the total national health care programme. In any case, the troubles between Stone and the top brass at HEW (of which the NIH is officially a part) are symptomatic of the widespread disquiet within the biomedical research community over the administration's policies for the NIH.

The disquiet stems chiefly from the

fact that the autonomy which the NIH has traditionally enjoyed has been considerably eroded in the past few years. Until the late 1960s there was little scrutiny of the NIH's programmes either by congress or the rest of the administration, but in the past couple of years, there has been a move to exert considerable management control over the NIH from Edwards's office in HEW. In addition the budget for biomedical research has levelled off and there has been considerable redistribution of funds from several areas of research into the politically sensitive cancer programme.

But the chief bone of contention came early last year when the administration proposed to scrap the NIH's training and fellowship programmes. Dr Robert Q. Marston, Stone's predecessor as Director of the NIH, was in fact fired when he resisted that proposal. After that, the administration let it be known that it was considering some radical surgery on the NIH's peer review system. Although both those moves have since been thwarted, largely because of loud protestations from biomedical scientists, Stone's forced departure is being viewed as yet another move towards tighter political control over the NIH from bureaucrats in HEW.

In an unrelated move, Dr Edwards himself resigned from the government to take up a post in industry a week after Stone's resignation was made known. Two top health posts are therefore vacant and the biomedical research community is anxiously waiting to see who will fill them.

University of Ghana celebrates jubilee

DURING the week (November 30 to December 7) the University of Ghana, situated in the Accra suburb of Legon, held its silver jubilee celebrations. In true academic style, the university had arranged lectures and exhibitions as well as two graduation ceremonies—one for the normal crop of last year's graduates and one for the presentation of honorary degrees to distinguished luminaries, including Dr Conor Cruise O'Brien, former Vice-Chancellor of the university.

The silver jubilee celebrations at Legon, like the recent silver jubilee celebrations at the Nigerian University of Ibadan, are certainly justified, for both universities have played a vital role in producing the trained manpower needed to run a newly emergent state. Sometimes the training process has not run particularly smoothly. Legon's celebrations were actually to have been held

last March but student demonstrations led to a closure of all three Ghanaian universities and to the cancellation of the original silver jubilee celebrations for Legon. Dr Alex Kwapong, the University of Ghana's skilful Vice-Chancellor has managed to keep the situation quiet this academic year, however and the university's stock has risen appreciably since March. Kwapong is a masterful negotiator and diplomat whose leadership and individualistic style, based on the groundwork laid by his predecessors, have helped to secure international prestige and financial aid for the campus.

Although all Kwapong's subordinates may not have his flair for administration, there has been significant growth in the university. The University of Ghana Medical School, which simultaneously celebrated its tenth anniversary, recently started dental and post-graduate courses. In addition, various other programmes such as computer science, journalism and population dynamics have all been set up within

the past two years.

The University has, in the past, been fortunate in attracting distinguished academics and promising young graduates to fill its teaching and research posts. This is, in part, a result of the generosity of the British, Canadian and Dutch governments who provide finance and high level manpower through technical assistance schemes. It is difficult to find Ghanaians of the appropriate calibre to man the nation's universities. The pecuniary rewards overseas, especially in the United States, are so enticing that Ghana, in common with all developing countries, suffers from a severe brain drain.

Overall the university academic staff is 75% Ghanaian but this proportion varies greatly from faculty to faculty.

In the scientific disciplines, where 41% of the professors and lecturers are non-Ghanaians, the loss of promising Ghanaians is most acute. Their absence abroad is most unfortunate, since the high quality of Legon's scientific research was documented in a recent study of the faculty of science funded by the Ford Foundation. The Ford Foundation's report also suggested a vigorous infusion of money and equipment to maintain the existing high standards. Unfortunately, in the present bleak economic climate, no benefactor has as yet been found to fulfil the committee's recommendations.

In a shrewd display of foresight, the Ghanaian government launched "Operation Feed Yourself" in 1972 in an effort to achieve self-sufficiency in food production. The faculty of agriculture met this challenge by expanding its research programme to improve the varieties of tropical rice and sugar cane being grown in Ghana. As a peripheral benefit the expanded production on the research farms has helped both the university, which can now economise by feeding its students some home-grown agricultural produce, and the Legon housewife, who can now buy hitherto unobtainable goods such as pasteurised milk, frankfurters and liver sausage.

Contemplating Legon's success in the past twenty-five years leads to great hopes for the next twenty-five. It is no mean achievement to teach well and to carry out good quality research in a developing country subject to an acute shortage of foreign exchange delays in imports, low indigenous salaries, a rate of inflation of 20% and a lack of technical staff, and where the whole economic climate revolves around the vagaries of a single agricultural commodity—cocoa. The ability to surmount these obstacles speaks well for the dons at Legon who, in the words of the university's motto, have achieved "progress with integrity". □

SIR,—A letter from Gerschenfeld and others concerning the problems of Chilean scientists under the military rule (*Nature*, March 29, 1974) has been answered by Eyzaguirre (*Nature*, July 5, 1974). I feel it my duty to present the view of a Chilean scientist, a witness of the entire process who works in Chile and intends to stay.

Since I do not think *Nature* to be the proper place for a political argument I shall only say that I disagree entirely with Eyzaguirre's interpretation of what happened in Chile. But neither can I accept his statements of facts. I do not deny the data—which refer to the Catholic University—the problem being rather that he would have others believe that the case of the Catholic University is representative of the Chilean universities. Without saying so explicitly, Eyzaguirre conveys that impression by mixing data of restricted significance with a very general and in my opinion superficial assessment of the Chilean situation before, during and after the military takeover.

According to Eyzaguirre, in the Catholic University "only 3.25% of faculty members were removed after the coup". The information I have collected for the University of Chile shows that approximately 30% of the faculty members and 11% of the non-academic staff were dismissed after the coup. The significance of this information stands out when one considers that the University of Chile enrolls 50% of the university students compared with 8% enrolled by the Catholic University.

I have been unable to obtain reliable data concerning the State Technical University, where the military intervention was most drastic. As to the University of Concepción (12.3% of the student population) the information I have points to at least 30% of faculty members dismissed. This figure is also a fair estimate for the Austral University (2% of the student population). It has been asserted that the worst hit have been the social sciences, but the private Technical University Santa Maria (3.3% of the student population) lost about 25% of its faculty members. I do not have dependable information on the Catholic University of Valparaíso (5.2% of the students) and the University of el Norte (4.2% of the students).

To sum up: the universities that enrol 75.6% of the students have lost about 29% of their faculty members since the military intervention, that is approximately 5,000 people, but dismissals still continue. As to the universities on which I have no consistent data (24.4% of the student population) the percentage of faculty members removed is presumably higher, consider-

ing that the State Technical University was entirely reorganised and is the second largest university in Chile.

The percentages of faculty members dismissed up to now are estimates based on various sources and might require some correction if reliable information is eventually disclosed to the public. Nevertheless, I trust that my estimates—especially those for the University of Chile—would stand a fair test and anyhow they clearly prove that the Catholic University is an exceptional case.

As to how many of the 130,000 university students have lost their

Letter from Chile

Dr Luis Izquierdo of the Faculty of Sciences at the University of Chile describes the situation now obtaining in the Chilean universities

places, dependable information is unavailable at this stage. On the one hand it is still uncertain how many might be allowed to register again; on the other hand, more than one year after the military intervention, students are still being expelled.

Eyzaguirre claims that faculty members removed from the Catholic University had been "engaged exclusively in different forms of political activity". Of course, political activity was not forbidden before the military intervention and nobody should be retrospectively condemned unless proven that he or she was exclusively engaged in these activities, since it is not the role of universities to support full time professional politicians either from the right or left. But, have there been thorough investigations?

It might be again the exceptional case of the Catholic University and hence the low percentage of faculty members removed. It is certainly not the case of the University of Chile in which faculty members were summarily investigated on the basis of secret denunciations. Charges were unspecified and all the defendant could do was to ask for certificates of good conduct from his less scrutinised friends. The procedure has been so irregular that in practice all decisions rested in the "fiscals" appointed by the military rector. I have witnessed much injustice, persecution and vindictiveness. I also must admit that some "fiscals" accepted their role in this legal farce so as to bring some help to their colleagues in disgrace.

In the State Technical University, University of Concepción and Austral University there was not even the

pretence of a legal procedure: the resolutions were based on black lists and faculty members or students were given notice through a standard form which specified no charges.

A decree from the government forbade universities and public institutions to employ faculty members dismissed, so those who had been engaged in research and teaching for years—notably in the basic sciences—had no alternative but to emigrate if they cared to persevere in their work. How can Eyzaguirre deride for practising *gauchisme de salon* those who left Chile and those lending them a hand from abroad? Of course there are people who have taken advantage of the international benevolence and claimed persecution when really looking for a better job, but they are exceptions and the overwhelming majority could not remain here because they had lost their jobs or felt threatened or could not endure the repressive environment.

The percentage of faculty members dismissed does not show in all its magnitude how serious the loss is for science in Chile. Many more scientists have left because of difficulties encountered in their work, such as disruption of research groups when senior investigators were dismissed, lack of research funds or of up to date libraries. The Chilean Society of Biology includes in its 414 members almost all the biologists at least partially engaged in research; 28% have left not to return under the present conditions. The situation of the Faculty of Sciences of the University of Chile is even worse. After struggling for 10 years we had succeeded in establishing it on a firm basis. Now, of the 123 faculty members in the department of Mathematics, Physics, Chemistry and Biology, 60 have left the country. Some would probably have been dismissed had they stayed, others lost hope of pursuing their research work at a satisfactory pace. Those of us who have invested so much of our time in the organisation of this Faculty, bitterly resent a set-back that takes us again to that state of dependence on foreign countries which we had sought to overcome.

The military intervention in Chilean universities has been up to now mainly repressive and has not achieved a new order. Recognised age-old vices of our university system persist in spite of the almost absolute power of present military rectors.

I have written this letter for the sake of truth to the best of my knowledge, convinced as I am that innuendoes, slander and lies from many quarters have corrupted Chilean political life in the last decade causing much individual misery and social distress.

news and views

Towards an anti-viral vaccine for a human cancer

from M. A. Epstein

WHEN the US National Cancer Institute set up the Special Virus-Leukaemia Program in 1964 (later expanded as the Special Virus Cancer Program) with the explicit objectives of (1) determining whether at least one human cancer is caused by an oncogenic virus, and if so of (2) developing an effective vaccine for the control of such a tumour (Rauscher, Carrese and Baker, *Cancer Res.*, **26**, 1176; 1966), the project was viewed with considerable scepticism by many leading workers in the cancer and virus fields. Looking back at the impressive progress made in tumour virology over the last ten years, the far-sightedness of the whole project is now evident, particularly in relation to the herpesviruses following striking recent advances with vaccines against some oncogenic members of the group.

Herpesviruses are known to cause the ubiquitous Marek's type of malignant lymphoma in chickens (Churchill and Biggs, *Nature*, **215**, 528; 1967; Solomon *et al.*, *Proc. Soc. exp. Biol. Med.* **127**, 173; 1968), an experimental malignant lymphoma in South American sub-human primates (Meléndez *et al.*, *Lab. Animal Care*, **19**, 378; 1969), and the Lucké kidney carcinoma of the leopard frog (Mizell *et al.*, *Science*, **165**, 1134; 1969; Naegele *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 830; 1974), and the closeness of the association of Epstein-Barr virus with African Burkitt's lymphoma and nasopharyngeal carcinoma in man is increasingly suggestive of some aetiological relationship (Epstein and Achong, *A. Rev. Microbiol.*, **27**, 413; 1973; Klein in *The Herpesviruses*, edit by A. Kaplan; Academic Press, London and New York, 1973; Henle and Henle, *Cancer Res.*, **33**, 1419; 1973). There is also a more tenuous association between herpes simplex virus type II and carcinoma of the human cervix (reviewed by Rapp and Buss, *Am. J. Path.*, **77**, 85; 1974), but this has been weakened by the failure of biochemical probes to detect the viral genome in tumour biopsy cells (zur Hausen *et al.*, *Int. J. Cancer*, **13**, 657; 1974).

The development in 1969 of live, attenuated herpesvirus vaccines capable of giving chickens almost complete

protection against Marek's lymphomas (Churchill *et al.*, *Nature*, **221**, 744; 1969; Okazaki *et al.*, *Avian Dis.*, **14**, 413; 1970) was of great significance both in economic terms and because it provided the first example of a naturally occurring malignant tumour to be controlled in this way. Although the importance of this step forward was widely recognised, its applicability to the ultimate control of those human cancers suspected of having a herpesvirus cause was clearly slight because of the impossibility of administering to man a suspected tumour-inducing virus, however attenuated. Indeed, it is unlikely that even an inactivated virus of this kind would ever be usable as a human vaccine because of the difficulty of proving total inactivation and the possibility that traces of the viral DNA in such a preparation might be capable of bringing about malignant transformation. But further progress with oncogenic animal herpesvirus vaccines is beginning to indicate the ways in which these difficulties could be overcome. Thus, it is now known that chickens can be significantly protected against Marek's lymphomas by vaccines free of virus nucleic acid. Lesnick and Ross (*Br. J. Cancer*, in the press) have reported some success with a vaccine consisting only of soluble viral antigens extracted from Marek's virus-infected tissue culture cells by treatment with nonionic detergent and other workers have used highly purified plasma membranes from similar virus-infected cells as a vaccine, and reduced the mortality from Marek's lymphomas by 94% when the vaccinated chickens were subsequently challenged with virulent virus (Kaaden and Dietzschold, *J. gen. Virol.*, **25**, 1; 1974).

Now Laufs and Steinke (this issue of *Nature*, page 71) have moved the problem closer to man by showing that sub-human primates can also be successfully protected against a herpesvirus-induced malignant lymphoma. These workers have used a combination of heat and formaldehyde to inactivate herpesvirus saimiri, which is otherwise carcinogenic in South American primates, and have shown by complement-fixation tests that the inactivated vaccine maintains consider-

able specific viral antigenicity after these treatments. Forty-two cotton-top marmosets have been immunised with the vaccine in the present experiments, have remained well after this, and have developed high titres of neutralising and complement-fixing antibodies to the virus. So far 22 immunised animals have been challenged with filtered, virulent herpesvirus saimiri in doses many times greater than those needed to induce fatal lymphomas in control animals and at the time of writing all were alive and well between 121 and 293 days after the challenge, whereas inoculated control animals died of malignant lymphoma within 52 days.

It seems reasonable to anticipate that herpesvirus saimiri vaccines free of viral nucleic acid will be developed before long on similar lines to those already being used to protect against Marek's lymphomas in chickens. The possibility this provides of a model system involving animals phylogenetically related to man has important implications for the planning, production, and testing of vaccines against suspected human tumour viruses. In this connection Epstein-Barr virus is not only a leading candidate because of its special association with Burkitt's lymphoma, but also has certain unique advantages. Thus, as has been pointed out elsewhere (Epstein and Achong, *A. Rev. Microbiol.*, **27**, 413; 1973), since Epstein-Barr virus causes infectious mononucleosis the efficacy of any vaccine developed could be tested by its ability to protect those at risk from this disease, and the existence of areas of high endemicity of Burkitt's lymphoma provide populations where the effect of such a vaccine on tumour incidence could be relatively easily tried out. Finally, since Burkitt's lymphoma occurs most frequently around the age of six, vaccination of infants in the months after birth should allow the efficacy of protection against Burkitt's lymphoma to be judged within five to 10 years. It seems that tumour control by an anti-viral vaccine will be the only way of proving whether or not Epstein-Barr virus is a human tumour virus aetiological related to Burkitt's lymphoma either with or without additional cofactors.

Circular polarisation in the Crab nebula

from R. D. Davies

THE Crab nebula—the remnant of the supernova of AD 1054—is a very remarkable object. It is unique in that it has been detected over the entire electromagnetic spectrum from radio through infrared and the visual to X rays and γ rays. Moreover the pulsar with the shortest known period lies at its centre. This pulsar holds the key to the source of energy in the Crab nebula which had been a mystery before its discovery. The pulsar is a neutron star rotating at 30 Hz. The important property of the pulsar in the context of the energy source of the Crab nebula is that it is slowing down—the rate of loss of rotational energy of the pulsar is equal to the energy radiated by the surrounding nebula.

The next stage is to understand the mechanism by which the pulsar supplies energy to the nebula. This energy is evident in the form of relativistic electrons and magnetic fields as these are required to produce the synchrotron emission from the nebula. But a simple estimate of the lifetime of the relativistic electrons which are responsible for the optical continuum emission indicates that they have a lifetime of only a few years. As a consequence such high energy electrons must be replenished continuously. This point was clearly recognised in the earliest work on the topic. Oort and Walraven in 1956 suggested that the moving wisps of gas seen near the centre of the nebula were evidence of continuing activity from a central star.

Recently Rees and Gunn (*Mon. Not. R. astr. Soc.*, **167**, 1; 1974) have looked afresh at the problem of the supply of relativistic electrons and magnetic fields to the nebula. They took a theory originally proposed by Piddington in 1957 and discussed subsequently by Kardeshev which had a central object responsible for winding up the magnetic field and thereby maintaining its strength. Rees and Gunn showed that the magnetic flux through the object increases in proportion to the number of rotations of the central pulsar; this indicates that as the nebula ages the magnetic field becomes increasingly important. The configuration of the field will be toroidal with an axis parallel to the spin axis of the pulsar. This axis is taken to lie along the major axis of the nebula (NW-SE). Linear polarisation measurements at optical and radio wavelengths show that the magnetic field is uniform and is essentially perpendicular to the major axis as would be expected from a toroidal configuration if the axis of rotation is in the plane of the sky.

Synchrotron theory indicates that more stringent tests can be made of the field configuration by measuring circular polarisation. Legg and Westfold (*Astrophys. J.*, **154**, 499; 1968) made a detailed analysis of the polarisation produced in the synchrotron mechanism and showed that a small component of circular polarisation is present. The sense of the circular emission depends on the inclination of the line of sight to the normal to the magnetic field, while the degree of polarisation is proportional to the square root of the magnetic field divided by the frequency. Such circular polarisation has already been detected in the radio emission from the magnetosphere of Jupiter and from several flat spectrum (that is, compact) extragalactic radio sources. It is now of interest to see if circular polarisation can be detected in the Crab nebula in the configuration suggested by the Rees and Gunn toroidal field model. Optical measurements by Martin *et al.* (*Mon. Not. R. Astr. Soc.*, **159**, 191; 1972) showed the presence of a weak circular polarisation but it is believed that this is produced by dust in the interstellar medium lying between the Crab nebula and the Earth.

No similar effect is produced by dust at radio wavelengths. An interesting new measurement of significantly higher sensitivity than made previously is now forthcoming. Weiler (this issue of *Nature*, page 24) reports the detection of circular polarisation in the radio emission from the Crab nebula at 1,415 MHz. Using the Westerbork Aperture Synthesis Radio telescope he finds a fractional polarisation of about 0.05% in four regions in the nebula. The circular polarisation is however of one sense only and does not show the change of sense in adjacent quadrants predicted on the toroidal model. Weiler concludes that the simplest magnetic field structure consistent with his observations is that of a relatively uniform field of 10^{-3} to 10^{-4} gauss with its line of sight component directed towards the Earth. On the face of it Weiler's detection is significantly above his measurement errors, although as he himself admits, independent confirmation would be useful at a lower frequency ($\lesssim 1$ GHz). If indeed this result is confirmed we are left with the need for a mechanism to maintain and amplify the magnetic field of the Crab nebula.

Models of nitrogen fixation

from A. J. Thomson

THE ability of primitive bacteria and some blue-green algae to reduce atmospheric nitrogen to ammonia at ambient temperature and pressure has long been a provocation to inorganic chemists. Until the mid-1960s, the only other known reaction of nitrogen at room temperature was the formation of metal nitrides either on clean metal surfaces or by lithium and alkaline earth metals. Considerable progress has been made in the isolation and analysis of the enzyme, nitrogenase, responsible for biological fixation (Hardy, Burns and Parshall, *Adv. Chem. Ser.*, **100**, 219; 1971). High purity preparations are now available from three genera of bacteria, including *Azotobacter vinelandii* and *Clostridium pasteurianum*. The enzyme, which requires an electron source such as ferredoxin or sodium dithionite, can be separated into two proteins, a high molecular weight component containing one or, less probably, two atoms of molybdenum and about 15 atoms of iron per molybdenum atom. The smaller protein contains two atoms of iron and two ions of 'labile' sulphide. Little is known about the environment of the metal atoms, although the high sulphur content of the protein

has led to the suggestion that sulphur is a ligand. There is no direct evidence that the metal atoms are directly involved in binding nitrogen during fixation.

The discovery by Allen and Senoff (*Chem. Commun.*, 221, 1965) of the first metal dinitrogen complex, $[\text{Ru}(\text{NH}_3)_5(\text{N}_2)]^{2+}$, during the attempted synthesis of $[\text{Ru}(\text{NH}_3)_6]^{2+}$ by the reaction of hydrazine with ruthenium trichloride trihydrate in water showed that N_2 could be made to ligand to metals. This was an important step since it had been a puzzling feature of inorganic chemistry that, whereas so many carbon monoxide complexes of low-valent metals were known, the isoelectronic N_2 molecule was apparently unable to bind. Since this observation some fifty complexes of dinitrogen have been reported (Sellman, *Angew. Chem. Int. Edit.*, **13**, 639; 1974). It is now a tacit assumption of all model studies that a metal ion, most probably molybdenum, is responsible for binding dinitrogen in nitrogenase.

The primary process of fixation, the trapping of the nitrogen molecule at ambient partial pressure, has now been successfully modelled by several groups using well defined compounds. A group

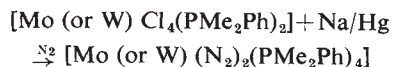
A hundred years ago



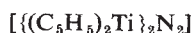
A RARE phenomenon, says the *Malta Times*, occurred in the forenoon of Monday, the 21st ult. During a strong wind from the south-west, which had prevailed for two days previously, the sea suddenly rose several feet and flooded the moles and roads surrounding the harbours, causing four or five steamers, moored between the Custom House and Calcara Rise, to snap their stern hawsers like packthreads, and carrying away boats that were hauled ashore in the French and other creeks. The sea then receded as suddenly as it rose, leaving portions of the bottom of the harbour exposed, upon which men and boys might be seen collecting fish and other marine animals that had been left aground by the retiring water. Shortly afterwards the sea resumed its ordinary level. Similar phenomena have been noticed occasionally during the course of many years.

From *Nature*, 11, 196, January 7, 1875.

from the Agricultural Research Council Unit of Nitrogen Fixation at the University of Sussex were first to devise a reaction based, appropriately, on a molybdenum complex (Bell, Chatt and Leigh, *Chem. Commun.*, 482, 1970).

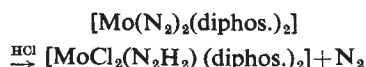


Subsequently, both Soviet and American teams succeeded in preparing



from gaseous nitrogen under mild conditions (Yu *et al.*, *Chem. Commun.*, 1178, 1972; Bercaw, Marvich, Bell and Brintzinger, *J. Am. Chem. Soc.*, 94, 1219, 1972; van Tamelen, Cretny, Klaentschi and Miller, *Chem. Commun.*, 481, 1972).

The second step required in the fixation process, the reduction of coordinated nitrogen to ammonia, has proved exceedingly difficult to achieve in any of the characterised complexes. Most attempts at reduction led to displacement of nitrogen. For example, solvolysis of $[\{(\text{C}_5\text{H}_5)_2\text{Ti}\}_2\text{N}_2]$ with methanolic HCl yields mainly nitrogen although ammonia and hydrazine appear as minor products. The first step in a well characterised partial reduction was achieved by Chatt, Heath and Richards in a reaction of their molybdenum (or tungsten) bis-dinitrogen complex with concentrated HCl (*Chem. Commun.*, 1010, 1972).



Now the persistence of the Sussex team has been rewarded. A report appearing in this issue of *Nature* (page 39) shows the room temperature reduction of *cis* or *trans*- $[\text{Mo(or W) (N}_2)_2(\text{PR}_3)_4]$ to two molecules of ammonia and one of nitrogen. The yields of ammonia go as high as 90% per nitrogen molecule reduced for a *cis* tungsten complex. Recently an interesting parallel development occurred in the United States, with the demonstration of the partial reduction of dinitrogen bound to a zirconium analogue of the cyclopentadienyl complex (Manriquez and Bercaw, *J. Am. Chem. Soc.*, 96, 6229, 1974). $[\text{C}_5(\text{CH}_3)_5]_2\text{ZrCl}_2$ undergoes reversible binding of nitrogen slowly at atmospheric pressure in the presence of sodium amalgam to form a binuclear species $[\{(\text{C}_5(\text{CH}_3)_5)_2\text{Zr}\}_2(\text{N}_2)_3]$. At -80°C , this product undergoes reaction with 10 M excess HCl in toluene releasing two molecules of nitrogen gas and forming a white solid, $\text{N}_2\text{H}_4 \cdot 2\text{HCl}$.

There is an interesting contrast between the British and American reactions. The former seems to involve a metal ion oxidation state change of +6 to supply the reducing equivalents to produce two molecules of ammonia from one of nitrogen. In the American scheme, however, a dimeric metal complex supplies only four reducing equivalents to lower the oxidation state of N_2 to N_2H_4 . It has been thought extremely unlikely that the six reducing equivalents could be supplied by a single metal centre. It is a basic tenet of the theory of electron-transfer reactions, borne out by experiment, that probabilities of simultaneous transfers of two or more electrons are very low and indeed, there is now a general doctrine of compulsory one-electron steps in true electron transfer (as opposed to atom transfer) reactions. If reduction is brought about by a cluster of metal ions, a dimer for example, this difficulty is partially overcome. Thus the zirconium complex apparently involves only a two-electron transfer per metal atom. Such electron transfer processes are well known although taking place sequentially. The fact that nitrogenase contains a large number of metal ions lends some credence to the view that a multi-metal centre is responsible for reduction. A somewhat similar hypothesis persists for the terminal oxidases which supply four electrons for the reduction of oxygen to water. The presence of a number of metal redox sites in the enzymes again suggests a cooperative interaction between the centres to supply the necessary reducing equivalents without any one centre undergoing a large change in oxidation state.

For these reasons the claim by the Sussex group to have shown "that molecular nitrogen can be reduced at a single metal centre in a protic medium"

is important. It is clear that, according to the overall stoichiometry of the reaction, each metal ion supplies six reducing equivalents. But since no kinetic or mechanistic evidence is presented in this report, it is surely not yet possible to conclude that reduction has gone on at a single metal centre. The low oxidation states of molybdenum and tungsten are well known for a tendency to form clusters. A recent report (Cotton, Frenz and Webb, *J. Am. Chem. Soc.*, 95, 4431, 1973) describes the structures of two dimers of molybdenum, $[\text{Mo}_2(\text{SO}_4)_4]^{3-}$ and $[\text{Mo}_2(\text{SO}_4)_4]^{4-}$. Apparently sulphate ion, the medium used in the Sussex experiments, can bridge two molybdenum ions. Professor Chatt and his colleagues have themselves suggested that the difference between the susceptibility to solvolysis of the diphosphine and monophosphine complexes may reside in the lability towards substitution by sulphate ion of the latter ligand. In addition, they note the escape of one molecule of nitrogen on mixing the reagents. Thus, consideration should be given to the possibility that metal clusters are involved at some stage in multi-step reduction of nitrogen. Some mechanistic studies will clearly be necessary to resolve this problem. Although one may start a reaction with a "well-defined complex" and be able to write down the overall stoichiometry not all the intermediates are thereby defined. Undoubtedly these latest two reactions are going to provide useful models for exploration of the mechanisms and the intermediates by which multi-step reductions can occur.

Future for thin films

from A. G. Holmes-Siedle

THE table shown on page 9 was composed to stimulate discussion on the limitations and requirements for electronically active thin films when used for making solid-state devices. At present, almost all such devices are dependent on the use of major circuit elements made from single-crystal ingots (for example, most thin-film hybrid circuits contain a silicon transistor chip). The crystal must be grown with immense care, then sliced, polished, diffused and diced. More than 50 processing steps may thus be involved before a minute 'chip' with the required electronic functions is produced. The chip then has to be attached to a header and contacted by thin wires. Techniques are now available to produce many of the same electronic functions in the same volume of material using only thin layers which are deposited on an inert surface and then etched into complex patterns to form minute active circuit elements (see, for example, Maissel, *Handbook of Thin Film Technology*; McGraw-Hill, 1972).

Table 1 Twenty-two electronic devices which could be made entirely of thin films

A Most active material is semiconductor		B Most active material is dielectric
Bipolar transistor	Magnetoresistor	Variable-threshold transistor
MIS transistor*	Photoconductive photosensor	Dielectric filamentary switch
Schottky-barrier transistor	Mechanical transducer	Pyroelectric detector
Rectifier	Chalcogenide switch	Light-beam deflector
Junction photosensor	Chalcogenide memory	Light-beam modulator
MIS electroluminescent diode*	Chalcogenide triode	Storage photosensor
Solar cell	Photostructural switch	
Charge-coupled device	Cold-cathode electron emitter	

*MIS: Metal-insulator-semiconductor

The main technological skill which is still lacking is the ability to deposit films of the required crystal perfection for the performance of certain crucial electronic functions. In conventional single-crystal electronics, effects such as minority-carrier transport, avalanche multiplication, tunnelling, acoustic surface-wave transport are commonly employed to amplify or modify the electrical signal entering the device. If these processes are depressed by the various forms of disorder and defect state found in the thin-film form, then this device principle cannot be embodied in a structure made entirely of thin films. For example, surface acoustic waves (SAW) cannot be transmitted along an evaporated silica film because the film is glassy and hence is not piezoelectric. The SAW signal-processing device therefore does not appear on the table, though it would merit a place if current attempts to make oriented piezoelectric thin films of zinc oxide to serve the same purpose as the conventional quartz chip are successful.

Grown or deposited films may be completely amorphous (see, for example Le Comber and Mort, *Electronic and Structural Properties of Amorphous Semiconductors*; Academic Press, 1973) or may contain very large numbers of grain boundaries, dislocations or point defects. Whether a device should be fabricated by thin-film or single-crystal technology usually depends on the feasibility of controlling the disorder and defect content of the thin film to the required degree and the profitability of doing this on a mass-production scale. That the stakes are high is illustrated by Westinghouse's recent announcement of a flat all-thin-film display panel, measuring about 11 inches diagonally (released at the Institution of Electrical and Electronic Engineers International Electronic Devices Meeting, Washington, 20 October). This device ("The World's Largest Integrated Circuit") is a laboratory product, paid for by the military. Yet a form of it could appear in everyone's living room within a few years, displacing the bulky eye of the television cathode ray tube.

Other concerns are working on similarly attractive, "all-thin-film" possibilities such as the pocket television

camera, the pocket optical computer and the optical waveguide to replace electrical wiring. Some items in the table are well developed commercial devices which are now normally fabricated from ingots; others are still in developmental or conceptual stages. Some further details on the criteria used in making this list may be found in my paper in *Rep. Prog. Phys.* (36, 699; 1974), which deals with the special case of amorphous materials but employs a suitable analytical approach for discussing thin films in general. Note that it is not only semiconductors which must be considered: increasing use is now being made of electronically active dielectrics. Though the single-crystal approach will never be completely displaced, it seems certain that thin-film techniques for electronically active materials have an important future and active discussion between physicists and technologists can only serve to promote the best use of those techniques.

Volcanic tephra on Crete

from Peter J. Smith

EARTH scientists or archaeologists can sometimes obtain information of mutual interest about the past. For example Ninkovich and Heezen (*Colston Res. Soc. Papers*, 17, 413; 1965) concluded from their studies of Mediterranean deep sea cores that tephra from violent volcanic eruptions must have fallen on Crete at least twice in prehistoric times. The older eruption took place more than 25,000 years ago and scattered material throughout the whole island. In the more recent fall, thought to be related to the eruption of Santorini (Thera) about 3,500 years ago, tephra (the so-called Minoan tephra) apparently covered the eastern half of Crete to an average depth of about 10 cm.

At first sight, the new volcanological evidence relating to the more recent eruption seemed to support Marinatos's (*Antiquity*, 13, 425; 1939) earlier hypothesis that the eruption of Thera led directly to the sudden collapse of the Minoan civilisation. But the picture became cloudier when Olausson (*Opera Botanica*, 30, 29; 1971), who also studied deep sea cores, claimed that the

younger tephra identified by Ninkovich and Heezen really comprised three or four layers of different ages, the youngest being not less than 5,000 years old. There is also a problem at the field level in that many archaeologists have found no volcanic ash on Crete at all, and thus remain unconvinced that tephra from the Thera eruption even reached the island.

Moreover, the general archaeological background is not as clear as it might be, for whereas the general destruction on Crete apparently occurred towards the end of the Late Minoan IB stage in about 1450 BC, or 1470 BC at the very earliest, the ruins on Thera itself have so far yielded only one sample of pottery typical of that stage. This strongly implies that there was a gap of several tens of years between the destruction of Thera and that of Crete. But geologists have generally ruled out the idea of two violent paroxysms, pointing out that upheavals which could have produced the pumice observed in the area would hardly have been able to occur within thousands of years of each other. So if there was only one catastrophic eruption, did it destroy Thera (in which case it could not have been the direct cause of the destruction of Crete tens of years later) or did it destroy Crete (in which case archaeologists need to discover why Thera was abandoned tens of years before the eruption)?

A more thorough search for, and study of, Cretan tephra was clearly necessary and has now been made by Vitaliano and Vitaliano (*Am. J. Archaeol.*, 78, 19; 1974). But, first, what are the possible sources of tephra on Crete? There are five Aegean volcanoes with a late Quaternary history of violent explosive eruptions; but three (Nisyros, Yali and Kos) lie to the east-north-east of Crete and are thus considered unlikely sources because the high-altitude dust-transmitting winds in the region are predominantly from the west. This leaves only Melos and Santorini (Thera) to the north. Volcanic material from particularly violent eruptions can however travel very long distances. Some Cretan tephra could therefore come from as far away as Italy; and the 5,000 year old eruption mentioned by Ninkovich and Heezen is now thought to have been Italian.

The question that Vitaliano and Vitaliano set out to answer is thus as follows: can Thera tephra actually be found on Crete, identified as coming from the Minoan eruption of 3,500 years ago, and related to Minoan archaeological levels?

The question became one of the refractive index of glass. On Santorini the tephra overlying the Thera ruins largely comprises volcanic glass with a

refractive index of 1.509. By contrast, the glass from a previous Thera eruption 16,000–17,000 years ago has a refractive index of 1.514, that from Nisyros (though similar chemically to that from Santorini) has an index of 1.502, that from Melos has an index of 1.505 and that from Yali has an index of 1.494. Composition without optical properties is apparently not suitable for the purpose of determining the source of tephra, although a combination of composition and refractive index should be slightly better than index alone.

And tephra with glass of refractive index 1.509 is indeed found on Crete. Vitaliano and Vitaliano have now discovered the Minoan tephra in trace amounts in over 50% of soil samples collected along the eastern section of the north coast. Traces of the tephra are also found at various archaeological sites, though so far never in circumstances thought to represent undisturbed remnants of the original Minoan ash. A few particles of the 25,000-year-old Italian glass (index 1.52) were also found, but none from the 16,000-year-old glass of Thera.

Unfortunately, the quantities, locations and arrangements (with respect to archaeological artefacts) of the Minoan tephra are as yet insufficient to enable firm conclusions to be drawn about the relationship between the eruption and the destruction of Thera and Crete. But having proved the potential of their method, Vitaliano and Vitaliano tentatively conclude that "little or no time elapsed between the abandonment of Thera and the fall of tephra on eastern Crete". Thus the preliminary volcanological evidence suggests that the general destruction of Crete was not a direct result of the Santorini eruption.

The mating game

from our *Animal Ecology Correspondent*

THERE is no doubt that male and female animals play the mating game according to somewhat different rules. Males are usually much more showily dressed than females and are given to attention-seeking displays. Often these take place when there are no females in sight and so cannot be regarded as being for the benefit of the opposite sex. Darwin in 1871 (*Descent of Man and Selection in Relation to Sex*; Murray, London) stated that males competed among themselves for mates while females did not compete but exercised some choice in mate selection. In a thesis which received wide publicity in 1962, Wynne-Edwards stated that males displayed amongst themselves to assess their own density and decide which of them should breed and which should not (*Animal Dispersion in Relation to Social Behaviour*; Oliver

and Boyd, Edinburgh). The decisions are reached through competition for such prizes as a territory or a high rank in the social hierarchy. According to this thesis the fertilisation of ova is a secondary male role; the primary role is to ensure that the population is not allowed to exceed an optimum density above which habitat resources would be overstrained.

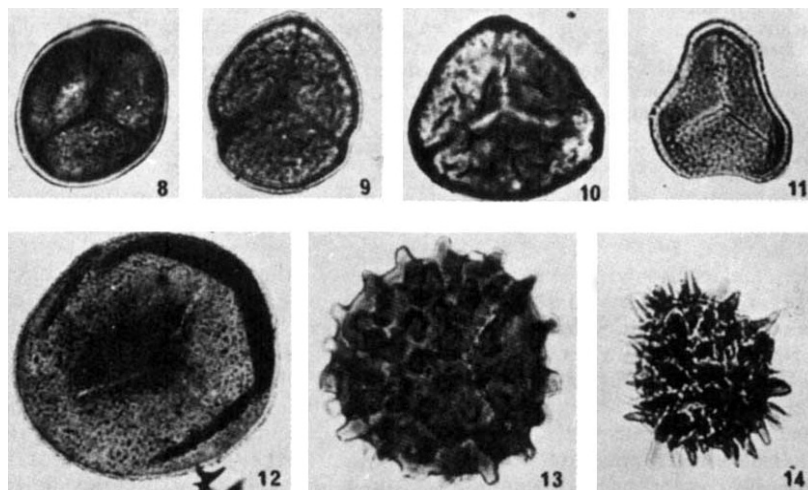
The late David Lack held firmly to the opinion that populations were kept below a level at which environmental damage would result by the action of predators or insect parasites (*Population Studies of Birds*; Clarendon Press, Oxford, 1966). In other words the size of the population is determined from without. Like Wynne-Edwards's, Lack's arguments are an extension of Nicholson's density-dependent control hypothesis (*J. anim. Ecol.*, 2, 132; 1933) inasmuch as there is a direct relationship between the severity of the controlling influence and the density of the population to be controlled. The displays which males put on apparently for other males, termed 'epideictic' by Wynne-Edwards, are seen by Lack to have nothing to do with a census but rather to have a sexual function in attracting females to males. In an early paper on black grouse (*Br. Birds*, 32, 290; 1939) he postulated that males displaying in groups attracted more hens than those displaying solitarily. He is thus critical of Wynne-Edwards's views that leks, traditional places for epideictic or nuptial displays, exist so that males can regulate the numbers of fe-

males fecundated and hence the population size.

Some long term studies of vertebrate leks are among the few studies of social behaviour in which observers have had the stamina to collect enough material to begin to assess these conflicting views.

The greater prairie chicken (*Tympanuchus cupido*) is a member of the grouse family which forms distinct leks. Robel and others (*Anim. Behav.*, 14, 328; 1966; *J. Wildl. Mgmt.*, 34, 306; 1970; *Auk*, 91, 75; 1970) have drawn attention to the fact that all matings take place at the lek. Between 1964 and 1969 there were few social upheavals at the chosen study leks and as a result 84% of the observed copulations were performed by dominant cocks. These also had the largest territories. This figure compares with 76% reported by Lumsden for dominant male sharp-tailed grouse *Res. rep. Ontario Dept Lands and Forests* No. 66, 1965 and 74% for dominant male sage grouse (Scott, *Auk*, 59, 477; 1942). During this long period of stability about 40 hens visited the lek each season of which on average, 32 were mated. In 1970 three, and in 1971 two dominant cocks were removed from the lek and with them went the stability of the lek. The number of social interactions observed each morning, formerly running at 23, rose sharply to 109 and the number of females mated fell to three and two. Social unrest amongst the males clearly reduced the number of young produced by limiting

Miospore sequence from Scotland



Dinantian miospores from the Spilmersford Borehole in East Lothian, Scotland. The description of 45 specimens examined in a recently completed survey provides one of the first palynological reference sequences for the Lower Carboniferous in Scotland. 8, *Retusotriletes incohatus* Sullivan; 9 and 10, *Pulvinispora scolecophora* sp. nov.; 11, *Waltispora planiangularata* Sullivan; 12, *Cyclogranisporites palaeophytus* sp. nov.; 13, *Raistrickia nigra* Love; 14, *Acoanthotriletes socraticus* sp. nov. In each case the magnification is 500X. Taken from the *Bulletin of the Geological Survey of Great Britain*, No. 45. (Her Majesty's Stationery Office, London, 1974.)

the number of females impregnated.

Elephant seals (*Mirounga angustirostris*) breed communally on selected small islands. Usually these are greatly overcrowded while other islands remain unburdened. In a six year study on Año Nuevo Island, California, Le Boeuf has focused attention on the small number of dominant males, usually fewer than one third of all resident males, which are responsible for the majority of copulations (*Am. Zool.* **14**, 163; 1974). Copulation frequency is directly related to success in competition between males for hierarchical accolade with alpha bulls being responsible for up to 49% of all matings. The Año Nuevo population is increasing, presumably due to subtle habitat changes, and with this increase is a decrease in the alphas' share of copulations. But there does seem to be some density dependent regulation in force. Le Boeuf in 1972 showed that a major source of pup mortality, up to 43% of all pups born, was the fighting of adult males (*Behaviour*, **41**, 1). Death to the pups was the result of being trampled upon by the bulls which weigh up to three tons. Crude though this mechanism may be, it is certainly effective. Social unrest among the males reduces the number of young produced by increasing infant mortality.

Some of the consequences of lek life in the Uganda kob antelope (*Adenota kob*) were reported in these columns a few weeks ago (**252**, 345; 1974). More than 15 years of study of the population in the Torro game reserve has revealed that there is a strong attachment to the home lek and adults of both sexes traditionally return home to breed (Buechner and Roth *Am Zool.*, **14**, 145; 1974). Only dominant males hold mating territories within the leks and, since clandestine matings are rare, they alone mate with females. There seems to be some limit to the number of territories in each lek, but what controls this is not known. Since rinderpest exterminated European cattle in this area kob populations have been increasing and a few additional leks are being formed. Population increase does not appear to be achieved by the establishment of additional territories within leks, but by the creation of additional leks.

Amid a welter of fascinating detail, these three studies reveal a couple of fundamental principles. First, they point out that Darwin's observations, *sensu stricto*, are incorrect because males fight not for females directly but for a conventional reward which itself confers the right to mate. Second, they show that social dominance is the key to conventional reward and hence to frequency of copulation. Additionally Robel's studies show that social stability between males is a major factor governing the number of females fecundated

and in Le Boeuf's study social interaction strongly influences infant survival.

Neither Lack's nor Wynne-Edwards's disquisitions can be proved or disproved by these studies although there is strong *prima facie* evidence that the behaviour of males has a regulatory function on the number of new individuals brought into the population. What has not been shown is whether leks serve primarily to census populations and their resources or to provide an attractive meeting, and mating, place. More long term studies may help to reveal the complications thrown in by such factors as overall population increase and so lead towards a correct interpretation of the function of leks. They may also, as a sideline help to indicate the area in which the regulation of human populations went awry.

Adapting to a chilly future

from Peter D. Moore

IF "The Threat of Ice" (see *Nature*, **252**, 216; 1974) is a real one, it may be salutary to learn how a variety of organisms adapt to cold. The adaptations of plants, which cannot migrate, must be physiological; they are also seasonal, in all but the most severe arctic and alpine climates. It has long been realised that the ability of many plants to tolerate cold varies with their recent temperature experiences (Levitt, *Handb. Pflanzenphysiol.*, **II**, 632; 1956); this knowledge has been used in the horticultural practice of frost hardening. Riedmüller-Schölm (*Flora, Jena*, **163**, 230; 1974) has recently examined the low temperature resistance of many Alaskan plants at monthly intervals

Another function for juvenile hormone

from our Insect Physiology correspondent

ONE attraction of the hormonal system of insects as a medium for the study of endocrinology has lain in the apparent simplicity of the range of hormones involved. As knowledge grows complexity increases. The diversity and subtlety of the effects of the juvenile hormone seem to militate against the formulation of any unitary theory of the control of metamorphosis. But a new factor has come to light in the course of studies by Nijhout and Williams (*J. exp. Biol.*, **61**, 481 and 493) on the tobacco hornworm *Manduca*. Throughout the early larval stages of *Manduca* there seems to be a continuing cycle of secretion of the brain hormone (the prothoracic-tropic hormone: PTTH) which induces the discharge of ecdysone by the prothoracic gland and of juvenile hormone from the corpus allatum, which ensures the maintenance of larval characters. But in the fifth or final larval stage the scene changes: feeding during this stage is more prolonged and when moulting does take place the larva suffers metamorphosis to the pupa.

One thing can be predicted from existing knowledge: the brain must not release PTTH while a large amount of juvenile hormone is circulating, or another larval stage will be produced. What is peculiar about the final larval stage, according to the results of Nijhout and Williams, is that the high titre of juvenile hormone inhibits release of PTTH by the brain. But when the larva reaches a certain weight (about 5 g) the corpora allata ceases

to secrete the juvenile hormone. Within about 24 h the juvenile hormone is cleared from the haemolymph and this results in the brain being rendered competent to release PTTH during the ensuing photoperiod. Growth continues for several days to give a final weight of about 8 g. The gut contents are then discharged and the behavioural and developmental changes that lead to pupation are set in train.

These observations provide an informative description of the hormonal changes during the last larval stage in Lepidoptera; and they illustrate a new function for the juvenile hormone. The failure of the normal arrest in juvenile hormone secretion is apparently responsible for the developmental arrest or diapause in the mature larvae of some Lepidoptera. Fukaya and Mitsuhashi and more recently Chippendale and Yin have stressed the importance of juvenile hormone in inducing larval dormancy. The onset and persistence of diapause in mature larvae can now be accounted for by a functional failure of the normal mechanism which inactivates the corpora allata of non-diapausing larvae.

The turning off of the corpus allatum secretion not only permits the morphological change of metamorphosis; it now seems to set in motion in caterpillars the endocrine events that lead to the moult itself. But what turns off the corpus allatum? Some environmental stimulus? Some proprioceptive stimulus? Or the passage of time?

through the course of a year and has found, rather predictably, that the highest degree of tolerance occurs in midwinter, though many species have a secondary peak of resistance at the end of winter, associated with the melting of snow cover. During these frost-hardy periods most plants examined (including angiosperms, gymnosperms, pteridophytes, bryophytes and lichens) were able to withstand a temperature of -80°C , in some cases for several days. Since the lowest observed temperature in the field during this time was -52°C the adaptations seem to be quite adequate for survival.

Perhaps surprisingly, maximum heat resistance was found to coincide with maximum frost tolerance in midwinter. For mosses these maxima ranged between 48 and 60°C , for vascular plants from 56 to 72°C and for lichens between 66 and 74°C . These figures exceed the published heat tolerance limits for plants of any climate, including tropical ones.

These data are all the more remarkable when one considers that many arctic and alpine plant species seem to be limited in their geographical distributions by high summer temperature (see Conolly and Dahl in *Studies in the Vegetational History of the British Isles*, edit. by Walker and West, page 159; Cambridge University Press, 1970). There are two points, however, which may help to explain the seeming anomaly. In the first place, high temperature resistance is very much less marked in summer than in winter; in the second place, temperature tolerance does not necessarily ensure that a plant would survive long periods at fairly high temperatures. Billings and Mooney (*Biol. Rev.*, **43**, 481; 1968) concluded that arctic/alpine plants have considerably higher respiration rates than do most temperate species. It could prove difficult for such plants to maintain a positive carbon balance at prolonged high temperatures.

The winter peak in high temperature tolerance must be regarded as a side-effect of the very physiological changes which provide low-temperature resistance. This has been observed previously, as has the drought resistance of such frost-hardy plants. The mechanism underlying these changes has recently been sought in cell membranes (see, for example, Raison, *Symp. Soc. exp. Biol.*, **27**, 485; 1973) particularly in mitochondria from fruit and vegetables. Wilson and Crawford (*J. exp. Bot.*, **25**, 121; 1974 and *New Phytol.*, **73**, 805; 1974) have now examined the level of unsaturated fatty acids in leaves during the development of low-temperature resistance. Using *Gossypium hirsutum* and *Phaseolus vulgaris* they demonstrated that the degree of unsaturation of the fatty acids associated with the

phospholipids (in the membranes of leaves) increased during the process of frost hardening. This was due in particular to increases in the percentage of linolenic acid in the phospholipids. It is suggested that the significance of this change is to lower the temperature at which a phase change occurs within these membranes from a liquid crystalline phase to a solid gel phase. A similar mechanism has also been suggested for poikilothermic animals. Such a membrane modification may have both a structural and a metabolic role by making membranes less susceptible to damage and allowing greater activity in certain enzymes.

Growth at 25°C also resulted in a higher degree of unsaturation in the phospholipid fatty acids, suggesting that this mechanism is also associated with high temperature tolerance. But tropical plants may have lost the capacity to increase the degree of fatty acid unsaturation in their phospholipids over short periods of time, thus rendering them sensitive to very low temperatures. As the next ice advance approaches, let us hope that we all have time to adapt.

Agglutination, proteolysis and cell division

from Robert Shields

WORKERS studying the control of cell proliferation have long been intrigued by the greatly decreased rate of DNA synthesis by normal cells in tissue culture when access to serum is restricted. When normal cultures reach their maximum (saturation) density the vast majority of the cells are in the G_1 phase of the cell cycle. Such cultures will undergo limited proliferation if the cells are treated with low doses of proteases or are given fresh serum. Under the same conditions tumour cells are not so subject to serum limitation; they will grow to far higher densities and are distributed throughout the cell cycle.

As tumour cells are more easily agglutinated by multivalent lectins such as concanavalin A (Con A) and confluent normal cells show transient increases in agglutinability when growth is stimulated with proteases, it was thought that exposure of lectin binding sites might regulate cell division. Experiments by Burger (*Nature*, **228**, 512; 1970) on transformed 3T3 cells with a preparation of trypsinised Con A (which was thought to be monovalent and so non-agglutinating) seemed to indicate that normal morphology and low saturation densities could be restored to the tumour cells by covering the Con A receptors on the cell surface.

A recent paper by Trowbridge and Hilborn (*Nature*, **250**, 304; 1974) has questioned these results; using the better characterised succinyl Con A (which is divalent) it was shown that covering the agglutination sites neither restored a low saturation density to transformed cells nor reduced DNA synthesis nor arrested cells in G_1 . These authors suggest that the restored saturation density seen by Burger may have been the result of cell death caused by the trypsinised lectin.

It is now realised that exposure of agglutination sites is similar in normal and transformed cells and that agglutinability requires rearrangement of these sites, which occurs more readily in transformed cells. It remained possible, however, that agglutinability *per se* was a sufficient condition for cell division until experiments with pronase demonstrated that density-restricted 3T3 cells could be made maximally agglutinable without cell division occurring (Glynn, Thrash and Cunningham, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2676; 1973).

A proteolytic activity on the transformed cell membrane seemed an attractive hypothesis in view of the similarities between transformed cells and protease-treated normal cells. The protease was thought to be responsible both for the high agglutinability and for an autocatalytic stimulation of division. Transformed cells were indeed found to have a greater proteolytic activity than their normal counterparts and moreover to have a proteolytic activity that converted plasminogen (present in the serum of the culture medium) to the protease plasmin. Reich and his co-workers (*J. exp. Med.*, **138**, 1056; 1973) showed that several of the characteristics of transformed cells such as altered morphology and the ability to grow in agar seemed to depend on plasmin activity. If these protease activities were really important in cell transformation the corollary was that inhibition of these proteases should cause phenotypic reversion of the transformed cells.

Once again early experiments looked promising. The growth of transformed cells was reduced and a lowered saturation was restored with protease inhibitors (Schnebli and Burger, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3825; 1972). With some protease inhibitors the cells showed a more normal morphology and had reduced agglutinability. But by this stage of the game it was becoming apparent that a lowered saturation density and morphological changes were poor evidence for restoration of density restriction of growth. A flurry of papers showed that inhibitors of plasminogen activation did not restore a low saturation density to transformed cells (Chou, Black and Roblin, *Nature*,

250, 739; 1974), and other protease inhibitors had no selective effect on tumour cell growth (McIlhinney and Hogan, *Biochem. biophys. Res. Commun.*, **60**, 348; 1974). Furthermore those that did arrested cells in the G₂ phase of the cell cycle (Collard and Smets, *Expl Cell Res.*, **86**, 75; 1974) and the cells continued to make DNA at their saturation densities (Schnebli and Haemmerli, *Nature*, **248**, 150; 1974). But these results only show that normal cell properties cannot be restored by certain protease inhibitors; they cannot rule out the possibility that some proteases are involved in maintaining the transformed state.

More antigens in hepatitis B

from Arie J. Zuckerman

AUSTRALIA ANTIGEN, now referred to as hepatitis B surface antigen in view of its close association with hepatitis B virus, was discovered as a lipoprotein which was immunologically distinct from normal low density lipoproteins. Examination of this antigen in the electron microscope revealed a remarkable morphological heterogeneity consisting of three principal virus-like particles (see Fig. 1). The main antigenic constituent is a small pleomorphic particle, 16–25 nm in diameter. A characteristic feature is the presence of tubular forms which vary greatly in length but with a constant diameter of 20 nm except for the bulbous swelling at one or both ends of the tubules. The third type of particle, the Dane particle, is also spheroidal measuring about 42 nm in diameter, with a 27 nm core, a 2 nm shell and an outer coat about 7 nm in thickness. There is now substantial evidence that the 42 nm particle is the human hepatitis B virus, the core being the nucleocapsid and the outer protein coat representing hepatitis B surface antigen. Immune electron microscopy and more recently various serological tests revealed the existence of two distinct antigen-antibody systems associated with the Dane particle; antibody to the core has a specificity entirely different from antibody against the outer protein coat. The morphological complexity of the particles associated with hepatitis B is matched by the complex antigenic reactivities of the outer coat. The antigenic constitution of the core is still under investigation.

The antigenic activity of the coat protein is associated with the small spherical particles, the tubular forms and the coat of the Dane particles. All these structures share a common group specific antigen (a) and the particles generally carry at least two sub-determinants: either d or y, which usually behave in a mutually exclusive

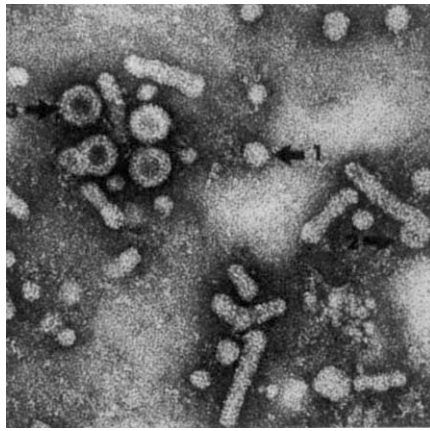


Fig. 1 Morphological appearance of hepatitis B antigen in serum showing three distinct entities: (1) Small pleomorphic spherical particles with a diameter of 16–25 nm. (2) Tubular forms. A terminal bulbous swelling is shown. (3) Double-shelled 42 nm Dane particles with a core and an outer coat. $\times 252,000$. (Electron micrograph reproduced with permission from A. J. Zuckerman *Hepatitis-Associated Antigen and Viruses*; North-Holland, 1972.)

manner although carried on the same antigen particle, and either w or r. There is evidence that the subtypes are the phenotypic expressions of distinct genotype variants of hepatitis B virus. Four principal phenotypes are currently recognised: adw, adr, ayw and ayr, but others are not precluded. Indeed complex permutations of these subdeterminants and new variants have been described, all apparently on the surface of the same physical particles. A remarkable geographical pattern of distribution of hepatitis B subtypes has emerged with four global zones, where there is an excess of one subtype, and regions where a mixture of subtypes is common (*Nature*, **247**, 2; 1974). These subtypes provide valuable epidemiological markers and offer a method for distinguishing one of several sources of infection. The surface antigenic reactivities do not seem to be associated with particular clinical forms of liver disease.

The above summary serves as an introduction to another antigen-antibody complex which is associated with molecules distinct from particles of hepatitis B antigen. Magnus and Espmark (*Acta path. microbiol. Scand.*, **B80**, 335; 1972) described a new distinct precipitating antigen, which they termed e, in sera containing hepatitis B surface antigen. This antigen differed markedly from the previously described determinants of the surface antigen. Paradoxically, antibody against e was found in serum specimens from healthy carriers of the surface antigen. The e antigen seemed to be somehow intimately associated with the pathogenesis of liver damage. Recently, Nielsen and col-

leagues (*Lancet*, **ii**, 913; 1974) reported their interesting observations on the e determinant. The e antigen was found by the simple immunodiffusion technique to be significantly more common in patients with chronic liver disease (chronic hepatitis and cirrhosis) with persistent hepatitis B antigenaemia than in patients with acute viral hepatitis. Furthermore, the e antigen seemed to be a valuable and important prognostic marker since progression to chronic liver disease was recorded by serial liver biopsies in 11 out of 19 consecutive patients with surface antigen-positive acute hepatitis associated with the e antigen. The clinical significance of the e antigen was supported by differences in the clinical, biochemical and histological findings between the patients with the e antigen and those without this antigen during the initial phase of viral hepatitis.

The exact nature of the e antigen is uncertain; it might be a host antigen produced by virus-infected liver cells or it might be related to another antigenic constituent of the infecting virus, perhaps the core of the Dane particle. Preliminary results from Nielsen's laboratory showed a large number of Dane particles in serum samples containing the e antigen, whereas e antibodies have previously been found in healthy carriers of the surface antigen in whom Dane particles had not been demonstrated. Though the precise relationship of e to hepatitis B virus is yet to be established, it is evident that the antigenic complexity of this unique infectious agent will continue to unravel.

Position effects in gene inactivation

from Benjamin Lewin

GENES translocated to positions close to heterochromatin seem in many species to become inactivated. Among the earlier observations were those made in *Drosophila* where, for example, some of the facets of the eye are white instead of red in heterozygotes in which the dominant gene has been transferred to a site adjacent to heterochromatin (see Lewis, *Adv. Genet.*, **3**, 75–115; 1950). It has since become apparent that this position effect variegation takes place in *Drosophila* when a wild type allele is placed in *cis* alignment with heterochromatin, with a recessive allele carried on the normal homologue; the wild type allele is inactivated in some cells, thus causing the variegation. The probability that a locus will be subject to this effect depends upon its relationship to the site of translocation; the closer it lies to heterochromatin, the more likely it is to be inactivated. Inactivation is de-

terminated during larval embryogenesis.

Although similar situations arise in the mouse, the rearrangements that cause the variegation always involve X-autosome translocations; in female mammals, one of the two X chromosomes, chosen at random, is inactivated in each somatic cell at any early stage of development, and the position effect can thus be shown only in those cells in which the rearranged X chromosome is inactivated to form heterochromatin. X inactivation occurs when the number of cells in the embryo is small, probably about 60 (Ohno *et al.*, *Cell*, **1**, 175-191; 1974; for review see Lewin, *Nature*, **249**, 9-11; 1974). This generates clones of cells in which only either the maternal or the paternal X chromosome is active, giving rise to variegation in the adult for any X-linked genes for which it is heterozygous. Thus although in an earlier study Cattanach, Wolfe and Lyon (*Genet. Res.*, **19**, 213-228; 1972) showed that the variegated coats of mice heterozygous for an X-autosome translocation display a pattern similar to that of chimaeric animals, the conclusion that the autosomal locus on the translocated X chromosome is inactivated in early development does not distinguish between random X inactivation and position effect variegation. An analysis which allows these phenomena to be resolved is now reported by Cattanach (*Genet. Res.*, **23**, 291-306; 1974).

The heterozygotes used in this study possess an X chromosome, X^T , that carries part of autosome 7; an X chromosome, X^N , that is normal; and a normal set of autosomes. The insertion within X^T carries the wild type genes $+^o$ and $+^p$ for coat colour; the normal copies of autosome 7 carry the recessive alleles c (albino) or its alternative c^{ch} (chinchilla) and p (pink-eye). Expression of the autosomal recessive coat colour alleles occurs in cells in which the insertion within X^T has been inactivated; coat colour genes are expressed by the melanocytes, which reach their ultimate positions in the hair follicles by migrating laterally down the foetal body. Each clone of melanocytes gives rise to a band across one side of the mouse coat; the clarity of these bands was enhanced in these experiments by use of the gene s , recessive spotting, which appears to reduce intermingling between cells of adjacent bands. Three sets of experiments were performed with this system.

In animals that suffered random inactivation, expression of the p and c^{ch} genes was followed in the bands. Three types of band were observed: $+^p +^o$ (wild type colour); $p c^{ch}$ (white); and $+^p c^{ch}$ (brown). The $+^p +^o$ bands are derived from cells in which the translocation chromosome X^T was active;

the $p c^{ch}$ bands represent cells in which X^T was inactive and both the $+^p$ and $+^o$ alleles on its insertion were inactivated. This confirms the clonal nature of the lines descended from cells in which either X^N or X^T respectively were inactivated. The third band, $+c^{ch}$, however, is derived from cells in which X^T was inactivated; but although the inactivation extended to $+^o$ it did not include $+^p$, which remained active. Since the $+^o$ locus is closer to the material of the X chromosome than is the $+^p$ locus, this is in accord with earlier experiments that showed that inactivation spreads sequentially from the X chromosome into the inserted autosomal material. By obtaining variegation of one of the inserted genes relative to the other, these results demonstrate that position effect variegation in the mouse is clonal and must have occurred early in development, between the time of X inactivation and the colonisation by melanocytes.

The animals used in these experiments had the genotype Xce^b/Xce^b , where Xce is a locus on the X chromosome which can influence the usually random pattern of inactivation; the Xce^b allele (previously known as Xce^1) permits random inactivation. But the Xce^a allele (previously described as Xce^c) causes the X chromosome carrying it to be inactivated more frequently in Xce^a/Xce^b heterozygotes; and in experiments in which the X^T chromosome carried the Xce^a allele (its normal homologue carrying Xce^b), the frequency of $+^p c^{ch}$ bands was increased, for the number of cells with $+^p$ active and $+^o$ inactivated was increased.

In the second set of experiments, random X inactivation was suppressed by using an X chromosome which carried Searle's translocation; this X is never inactivated, so it is the genes carried by the other X chromosome, in these experiments X^T , that fail to be expressed. But mice which carry $+^o$ on X^T and carry c on both copies of autosome 7 may still display some variegation for the c phenotype. Thus although X^T is inactive, the $+^o$ gene on the inserted autosome segment can still be expressed, albeit at frequencies less than 5%. The pigmented areas are always small—much smaller than whole bands. When the autosomes of such mice carry p as well as c , extensive pigmentation occurs in the production of $+^p c^{ch}$ regions, in which $+^p$ must be active in the inactivated X^T chromosome, although its $+^o$ gene remains inactive. The $+^p c^{ch}$ regions may occupy whole bands; this suggests that only genetic events are implicated in their establishment, excluding alternative explanations such as cell selection. By extension, this argument implies that the small pigmented areas in the mice where only the c locus is the sub-

ject of variegation also must have a genetic origin; so these must be derived either by late determination or by a reversal of an earlier inactivation.

In the third series of experiments, animals whose variegation had been examined were kept for up to 18 months and scrutinised for changes in coat colour as ageing took place. It has been observed previously that in general c areas uniformly darken with age. This suggests that a reversal of inactivation takes place with time, leading to expression of the $+^o$ gene on X^T . When the $p c^{ch}$ areas of the mice used in Cattanach's experiments were examined, he found that they first turn the brown colour characteristic of $+^p c^{ch}$ cells and then turn the black colour characteristic of $+^p +^o$ cells. This suggests that the inactivating influence along the autosome insertion within the X chromosome gradually retreats, causing sequential reactivation.

From these results, Cattanach proposes an alternative to the previously proposed explanations of position effect variegation in the mouse. The variegation has usually been attributed to a sequential inactivation proceeding along the translocated autosomal region from the X chromosome. This might involve structural changes, or, as has been suggested for *Drosophila*, the addition of suppressor molecules to the inactivated regions. Given that reactivation is responsible for events occurring later in development, however, Cattanach argues that perhaps only one mechanism is responsible for all variegation, irrespective of whether it takes place early or late. Thus after inactivation of the translocated autosome (possibly still to a limited extent), a progressive reactivation takes place with time from the point most distant from the site of translocation; this must vary in rate in different cells. If reactivation at any locus occurs at an early stage, an active gene will be clonally inherited; although the descendants of this cell all will possess this activity, subclones may be generated as progressive reactivation applies to other loci. This model has the advantage that the position effect can be explained by a single mechanism operating at all stages of development, in contrast with models where stability of the inactivated autosome region is implied and for which an alternative model must be invoked to explain reactivation. Since there is no suggestion that the reactivation applies to genes of the X chromosome itself, the model draws a clear distinction between the sex heterochromatin and the autosomal insertion, although no implications are made about the molecular mechanisms that may maintain the inactive X but progressively fail to inactivate the autosomal region.

articles

Unconformities in the sediments of the Indian Ocean

Thomas A. Davies & Oscar E. Weser

Deep Sea Drilling Project, Scripps Institution of Oceanography, La Jolla, California

Bruce P. Luyendyk

Department of Geological Sciences, University of California at Santa Barbara, Santa Barbara, California

Robert B. Kidd*

Institute of Oceanographic Sciences, Wormley, Surrey, UK

Deep sea drilling in the Indian Ocean has revealed unconformities of oceanwide significance. The unconformities are of Oligocene, early Tertiary and late Cretaceous age and can be explained as a consequence of climatic events in Antarctica and subsequent variations in the circulation pattern of the whole Indian Ocean.

UNTIL recently it was believed that sedimentation in the deep oceans is essentially continuous. One of the more unexpected results of the Deep Sea Drilling Project (DSDP) has been the discovery of large gaps in the sedimentary record at many sites. Major hiatuses of regional significance have now been identified in the Atlantic, Caribbean, Pacific, Antarctic and Indian Oceans. Data from sites drilled on DSDP Legs 22–27 and part of 28 (Fig. 1, refs 1–7) show that in the Indian Ocean gaps in the sedimentary record have been encountered in a broad spectrum of terrigenous, pelagic, biogenic and volcanogenic sediments encompassing late Mesozoic and Cainozoic time. The hiatuses were encountered at more than half the 48 sites and are developed on a wide variety of topographic features over a broad range of depths. They seem to show temporal groupings centred in the Oligocene, early Tertiary, and late Cretaceous.

Here we use the term unconformity to refer to a significant gap (demonstrated or inferred) in the stratigraphic record (disconformity or paraconformity). Unconformities are considered to represent active erosion or complete non-deposition of sediment. At many sites undated intervals were encountered, barren of microfossils, usually with a lithology of detrital or pelagic clay accompanied by zeolites and other indicators of extremely slow sedimentation. These intervals, which may be considered dissolution facies, represent slow sediment accumulation beneath the carbonate compensation depth (CCD). They may or may not contain unconformities, but this cannot easily be resolved because of the lack of biostratigraphic control. There is indirect evidence that in many cases the undated intervals do encompass unconformities.

The descriptions of unconformities from the results of some of the earlier legs of the drilling programme have been somewhat equivocal because of the often large unsampled intervals between cores. Fortunately, in the Indian Ocean programme,

continuous coring or instances of greater than 50% coring were frequent. So we have considerably more confidence in recognising unconformities and in determining their extent. The Oligocene unconformity can be recognised with certainty at 15 of the 33 sites sampling that time interval and there is good evidence for its presence at 11 further sites making a total of 26 out of 33 sites. Corresponding figures for the early Tertiary and late Cretaceous unconformities are 18, maybe 27, out of 30 sites, and 9, maybe 13, out of 13 sites, respectively. It is clear, therefore, that these unconformities are of oceanwide significance. We believe that they can be explained as the consequence of climatic events in Antarctica and subsequent variations in the circulation pattern of the whole Indian Ocean.

Stratigraphic record in the Indian Ocean

Western Indian Ocean. In Fig. 2 the stratigraphic intervals recorded at sites in the western Indian Ocean are shown projected into a north–south line (locations in Fig. 1). Unconformities and/or undated intervals are shown centred on the Oligocene and the early Tertiary (or latest Cretaceous). The crust in the western Indian Ocean is generally younger than the eastern basin, with most western sites being no older than latest late Cretaceous. Consequently, a separate late Cretaceous hiatus is recognisable at only a few sites. True unconformities were encountered at 11 sites. Undated intervals, generally represented by a dissolution facies of zeolitic clay, are equally common. In many cases, such as sites 248 and 250, the sediments in these intervals are thin relative to those above or below, implying that the undated intervals do contain true unconformities. Local unconformities are indicated since the late Miocene at Site 240 in the Somali Basin and in the Pliocene at Site 249 atop the Mozambique Plateau, as well as at sites in the north-western basins. The maximum duration of the Oligocene hiatus is from the late Cretaceous to the early or middle Miocene (Site 249), indicating that the physical causes of the disconformities and dissolution facies ceased operating in the early/middle Miocene but could have been initiated at any time prior to this back to the late Cretaceous. The time-span of the early Tertiary hiatus extends from at least the Maestrichtian (Site 248) to late Eocene (Site 239).

Generally it seems that the cause of these unconformities is pervasive throughout the water column since both shallow and deep sea floor have been affected.

*Present address: Deep Sea Drilling Project, Scripps Institution of Oceanography, La Jolla, California.

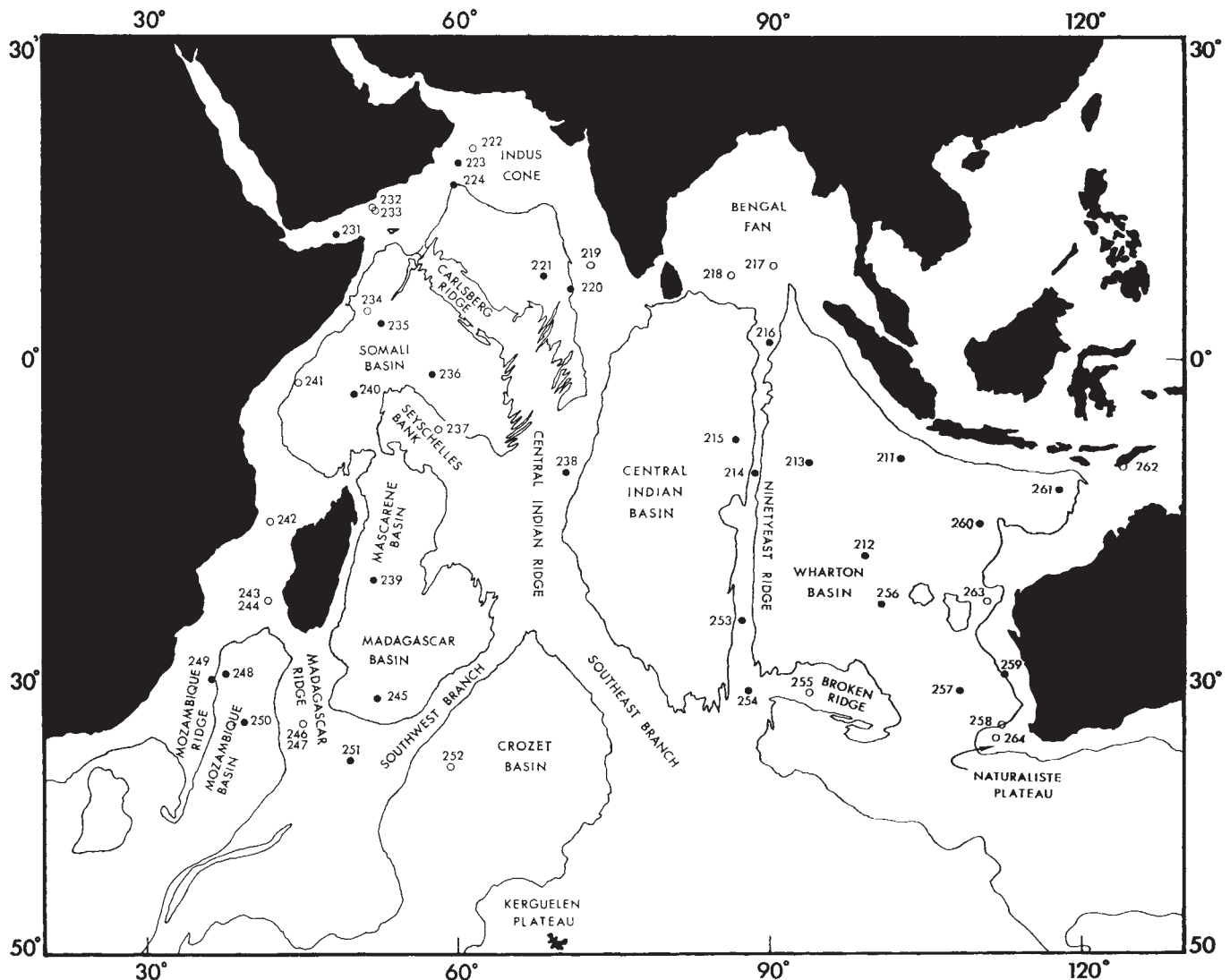


Fig. 1 Deep Sea Drilling Project sites in the Indian Ocean. ●, Sites which reached basement; ○, other sites. The principal physiographic features are defined by the 4,000 m contour.

Eastern Indian Ocean. Data from sites in the eastern Indian Ocean are interpreted to show hiatuses centred on the Oligocene, early Tertiary and/or late Cretaceous (Fig. 3). The upper limit of the Oligocene hiatus in Figure 3 is in the late Miocene or Pliocene (Sites 264, 258, 261, 215). The limits of the earlier hiatuses are not well defined but we interpret the upper limit of the early Tertiary hiatus to be in the middle Eocene (Site 216, 217) and the limit of the Cretaceous hiatus may be as young as late Palaeocene (Site 264).

Unconformities are again present in a variety of bathymetric features. They are found in the shallow sites on the Naturaliste Plateau, Ninetyeast Ridge and Broken Ridge and at deep sites such as 213, 215, 259 and 263. At the deeper sites, undated intervals—which may or may not include unconformities—are common.

Comparing Figs 2 and 3 we see that the hiatus centred on the Oligocene ends in the middle Miocene in the west and in the late Miocene/Pliocene in the east. On the Ninetyeast Ridge, the Oligocene hiatus ends in the late Oligocene, suggesting that if the same physical cause is responsible for both the shallow and deep hiatuses, it ceased functioning in the shallow regions first. The early Tertiary unconformity ends in the early Eocene in the west and middle Eocene in the east. The causes of both these unconformities seem to have ceased operating sooner in the west than in the east.

Regional extent of the Cainozoic hiatuses

The danger of regionalising these observed hiatuses cannot be overemphasised, considering our imperfect knowledge of the history of the local sedimentation regimes at each site. Nevertheless it is instructive to try.

In Fig. 4 we have plotted Oligocene and older DSDP sites from Legs 21 to 28 on a late Oligocene tectonic reconstruction. The extent of the Oligocene hiatus is indicated by the shaded regions. The hiatus is present at both shallow and deep sites whereas a complete Oligocene section (apparent) was recovered in the north-west Indian Ocean at shallower sites near the ancient ridge crest and nearer to the palaeoequator. Figure 4 shows that the western Pacific sites of DSDP Leg 21 exhibit the same hiatus. Kennett *et al.*⁸ described this hiatus as a disconformity found at various depths between 1,400 and 4,500 m. It seems to end in the middle Miocene, about the same time as in the western Indian Ocean. Kennett *et al.* pointed out that Oligocene unconformities are also found in southern Australia, New Guinea and parts of New Zealand⁹⁻¹¹. Carter and Landis¹² described the south Australian unconformity as a paraconformity below shelf limestones of early Miocene age. Oligocene hiatuses are not generally found in the central Pacific¹³. In the south central Pacific DSDP Leg 8 results show a disconformity near the late Eocene/early Oligocene but the Oligocene itself is marked by calcareous ooze with very high sedimentation

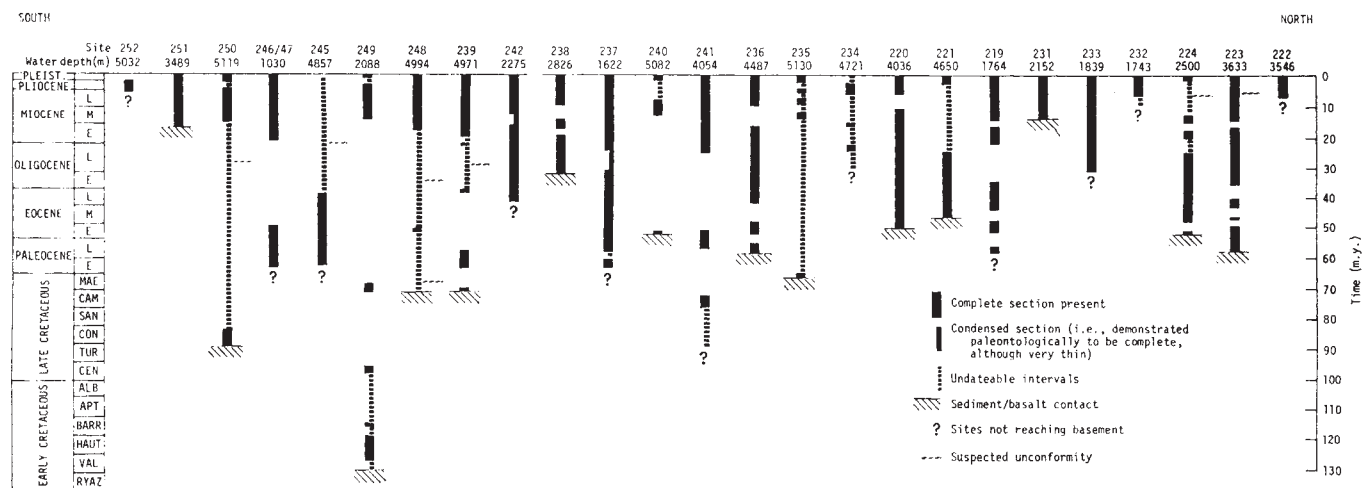


Fig. 2 Stratigraphic section sampled at sites in the western Indian Ocean. Sites 243 and 244 are omitted since no cores were recovered from these sites. Basement ages at Sites 248 and 249 were determined radiometrically⁴.

rates¹⁴. High calcareous sedimentation was also seen in the sediments sampled on DSDP Leg 3 in the South Atlantic¹⁵, where the Oligocene is represented by two chalk ooze formations. Clearly, erosion and/or non-deposition and dissolution in the Indian Ocean and south-west Pacific during the Oligocene was contemporaneous with high carbonate deposition in other oceans of the world.

In Fig. 5 we have plotted Indian Ocean and south-western Pacific DSDP sites on an early Eocene reconstruction. The early Tertiary hiatus is more difficult to regionalise because it is often obscured by the overlying Oligocene hiatus and at many sites the oldest sediments recovered were younger than Eocene. But certain patterns seem similar to those of the Oligocene data; these include uninterrupted sedimentation in the north-west, unconformities on shallow structures such as the Ninetyeast Ridge, and a well developed hiatus in southerly latitudes. The similarity in the regional patterns of the early Tertiary and Oligocene hiatuses implies that they may be due to similar causes.

The early Tertiary hiatus is documented in the central Pacific by DSDP Leg 17 results¹⁶. This hiatus is a disconformity which ends in the middle Eocene in both shallow (2,300 m, Site 171) and deep (5,500 m, Site 164) sites. The central Pacific hiatus ends at the same time as the early Tertiary hiatus on the Ninetyeast Ridge. Leg 21 results also show a hiatus ending in the middle Eocene in the southwest Pacific⁸ (Fig. 5). In the South Atlantic, the early Tertiary sequence is complete¹⁵.

Late Cretaceous sediments have not often been sampled by DSDP, but in the central Pacific some Leg 17 results indicate an unconformity ending in the Campanian as in the Indian Ocean.

It seems, therefore, that regional hiatuses in the Cretaceous and early Tertiary in the Indian Ocean are closely allied with contemporaneous ones in the central and south-west Pacific. The Oligocene hiatus in the Indian Ocean and south-west Pacific, however, occurred at the same time as high calcareous sedimentation in the central Pacific and South Atlantic.

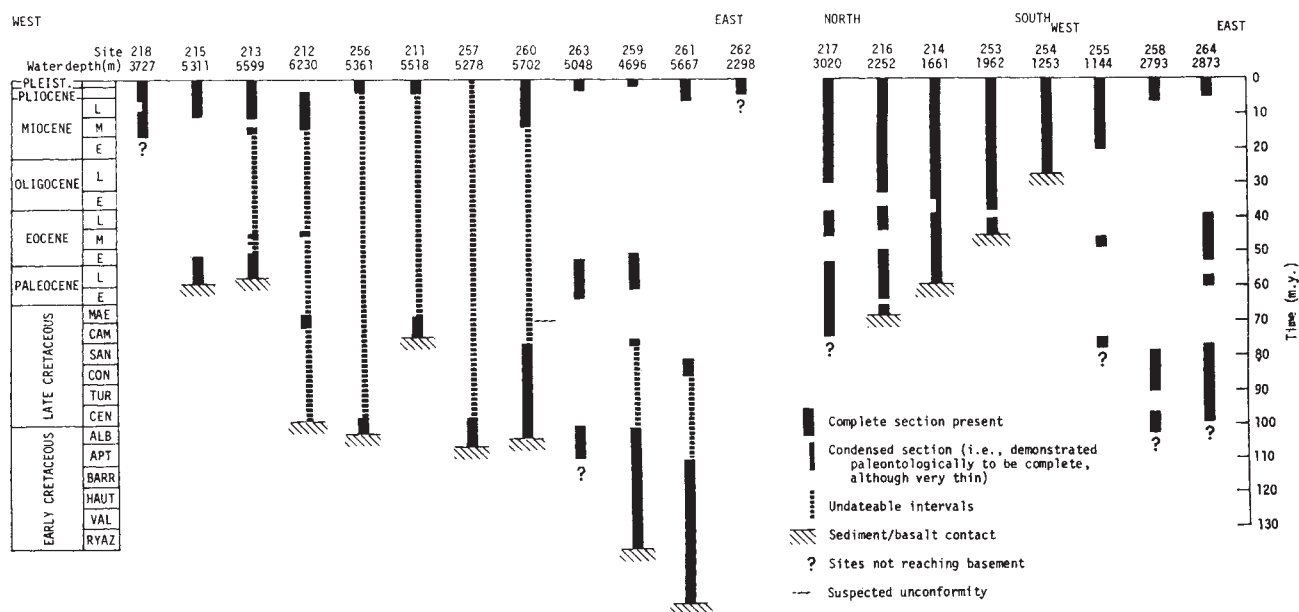


Fig. 3 Stratigraphic section sampled at sites in the eastern Indian Ocean. Ridge and plateau sites (right) are separated from the basin sites (left). The basement age for Site 212 is derived from geophysical evidence¹⁷.

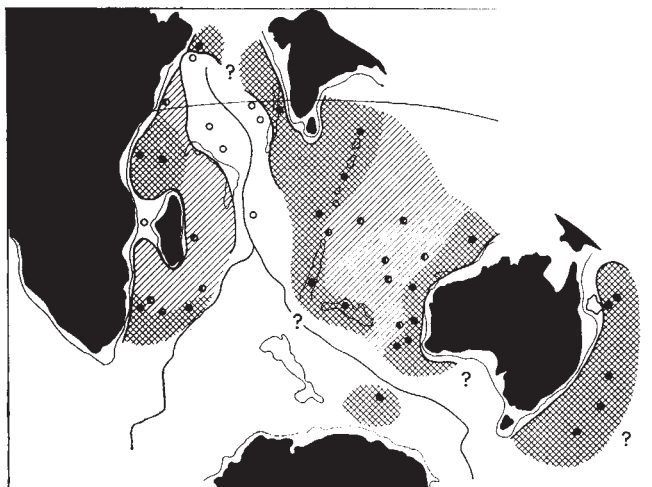


Fig. 4 DSDP sites plotted on an early Oligocene reconstruction of the Indian Ocean²⁶. Open circles represent sites where the sedimentary section is complete; solid circles where there is a proven unconformity, and half circles where there is an undated interval or an inferred unconformity. The shading indicates the extent of the Oligocene unconformity; crosshatching showing the extent of the proven unconformity.

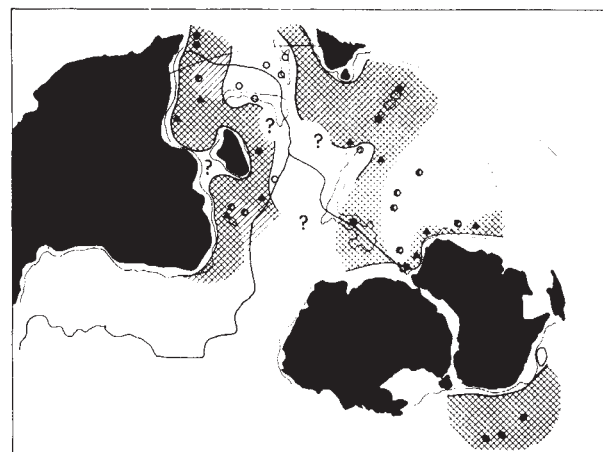


Fig. 5 DSDP sites plotted on a middle Eocene reconstruction of the Indian Ocean²⁶. ○, sites where the sedimentary section is complete; ●, sites where there is a proven early Tertiary unconformity; half circles where there is an undated interval or an inferred unconformity; ▲, sites where an early Tertiary unconformity cannot be separated from the overlying Oligocene unconformity. The shading indicates the probable extent of the early Tertiary unconformity; crosshatching showing the extent of the proven unconformity.

Significance of the hiatuses

Attempts to explain the cause or causes of these hiatuses must consider numerous variables, including tectonism and continental drift, world climate, oceanic productivity, and oceanic circulation and oceanic chemistry, all of which are inter-related in a complex way. In perhaps the simplest cases (the early Tertiary unconformity on Broken Ridge (Site 255) and those near Owen Ridge (Sites 223 and 224)) the unconformities are clearly the result of tectonic uplift and subsequent erosion^{2,17}. On the other hand Kent¹⁸ has drawn attention to the fact that hiatuses in the sediments of the south-west Indian Ocean, while seemingly corresponding to tectonic events on land, are more likely to have been the results of variations in oceanic circulation and sediment supply rather than being directly attributable to tectonic events. The same may be said of the gaps found in the sedimentary record on the Naturaliste Plateau; for the sites in the deep basins remote from tectonic activity the links between hiatuses and such activity must be even more tenuous, if not non-existent. Even if we exclude locations for which there is a clear case for a tectonic origin for the unconformities, we are still left with the task of explaining the occurrence of oceanwide gaps in the sedimentary record. Recognising the limited data available and the complexity of the problem, we will limit our speculations to the Oligocene and early Tertiary hiatuses.

The following observations might be considered facts regarding these hiatuses which require explanation:

- (1) Both hiatuses are expressed over a wide depth range.
- (2) Well developed unconformities appear in the western Indian Ocean and south-west Pacific whereas undated intervals, probably containing unconformities, are more common in the eastern Indian Ocean.
- (3) The early Tertiary and Oligocene hiatuses have similar regional extent in the Indian Ocean and south-west Pacific.
- (4) The Oligocene hiatus is not found in the central Pacific as is the early Tertiary hiatus.
- (5) The Oligocene unconformity in the Indian Ocean and south-west Pacific occurs in concert with high calcareous sedimentation rates in the South Atlantic and central Pacific.

In our discussion we use the following model for hiatus formation in the deep sea. True unconformities are due to erosion or non-deposition caused by vigorous surface and/or deep ocean currents. The fact that the hiatuses in question cover such a wide depth range indicates that both deep and surface circulation have been involved. Dissolution facies are

formed below the CCD where calcareous material is dissolved. The CCD is the level where dissolution of carbonate by cold, under-saturated bottom waters balances carbonate supply from surface waters. The CCD may be moved shallower by a decrease in regional productivity, removal of oceanic carbonate by precipitation on shelf areas, or an increase in the amount and rate of supply of cold bottom waters, all of which result in an increase in the under-saturation of bottom waters¹⁹.

Bottom water in the Southern Hemisphere forms in the Antarctic shelf regions, notably in the Ross and Weddell Seas²⁰. Weddell Sea water in part flows east into the south-western regions of the Indian Ocean where it behaves as a western boundary undercurrent. Bottom water also drifts north through fracture zones in the south-east Indian Ridge, where it may be joined by Ross Sea water flowing west between Australia and Antarctica, to pass through the gap between Naturaliste Plateau and Broken Ridge into the Wharton Basin²⁰. This pattern of circulation seems to mirror the regional development of the Oligocene hiatus and possibly the early Tertiary one as well, and would explain why the hiatuses are more definitely expressed in the western ocean regions. Tectonic and climatic events in the Antarctic region are therefore important.

An important event in the late Cretaceous was the rifting of New Zealand from Antarctica and the opening of the Ross Sea²¹. Shortly after this, in the early Eocene or before, climatic deterioration and glacial conditions occurred in Antarctica²². This combination of events may have led to the formation of substantial amounts of 'aggressive', erosive Antarctic Bottom Water, Ross Sea water entering the central and south-west Pacific to erode and dissolve sediments and Weddell Sea water spreading eastwards between Africa and Antarctica into the western basins and through the gap between Broken Ridge and Naturaliste Plateau into the Wharton Basin. Surface circulation was intensified as a result of climatic cooling and perhaps increased storminess. This could explain the occurrence of well-defined unconformities at many of the shallow sites contemporaneous with undated intervals and unconformities in the deep basins. If surface productivity and faunal diversity were also decreasing during the early Tertiary, as is widely believed, these would serve to enhance shoaling of the CCD and result in the extensive formation of dissolution facies.

A similar story can be constructed for the Oligocene hiatus since climate deterioration and late Oligocene glaciation are

probable for Antarctica at that time²². It is curious that the Oligocene hiatus is not found in the central Pacific. Post-Oligocene erosion is found in the Samoan gap, the bottom water conduit into the central Pacific²³. Dissolution of great quantities of carbonate in the Indian Ocean and south-west Pacific in the Oligocene is possibly related to the contemporaneous high carbonate sedimentation elsewhere in the Pacific and Atlantic as the ocean responded to the need to maintain a chemical balance. Also, it is possible that at this time Ross Sea water was confined to the south-west Pacific by Melanesia and thus had relatively little influence in the regions farther north.

Between the late early Eocene and the middle of the Oligocene climatic conditions in Antarctica were probably a little warmer. This was sufficient to reduce the supply of Antarctic Bottom Water, encourage increased surface productivity, and diminish the intensity of current activity to the point where sedimentation could resume. The Oligocene hiatus was brought to a close by the gradual establishment of the present pattern of circum-Antarctic flow sometime near the end of the Oligocene²⁴. This again reduced the inhibiting influence of Antarctic Bottom Water on sedimentation in the ocean basins farther north, since a substantial proportion of the Antarctic Bottom Water was diverted into circumpolar flow and proportionately less found its way into the basins.

This attempt to attribute regional patterns of unconformities to climatic events in Antarctica is incomplete in several respects. First, the present day pattern of Antarctic Bottom Water circulation and its effects on deep ocean sedimentation are not entirely clear, much less these effects 60 Myr ago. Palaeobathymetry models would be helpful, as would further hydrographic stations and piston cores in the Indian Ocean. Second, present understanding of what effect Antarctic glaciation had on ocean surface water movements is equally vague. These currents have probably contributed to forming the hiatuses at shallow sites. We recommend experimental and analytical study of these currents taking into account continental dispersion and palaeowinds²⁵. Finally, more precise evaluations of the stratigraphy at each site will result in a more refined picture of palaeocirculation patterns.

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Structure and assembly of filamentous bacterial viruses

D. A. Marvin & E. J. Wachtel

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520

The protein coat of filamentous bacterial viruses is constructed from segments of a helix that extend in an axial direction, and overlap each other like shingles or fish scales to form a hollow cylindrical shell. This hollow shell contains the viral DNA.

THE protein coat of filamentous bacterial viruses^{1,2} is rich in α helix. This has enabled us to develop a model for the virus structure. We have used low resolution X-ray diffraction data to define the general arrangement of α helices, followed by molecular model building to define atomic positions. Our model

has implications for the morphogenesis of these viruses, and also for the structure of linear assemblies of proteins in general.

Filamentous bacterial viruses consist of linear assemblies of coat protein subunits encapsulating single-stranded, circular DNA. The virions measure about 60 Å in diameter by 10,000 to 20,000 Å long, depending on the strain, and contain no more than 12% by weight of DNA. The major coat protein in all strains comprises about 99% of the viral protein, has a molecular weight of about 5,000, and is largely α helical. Their relative simplicity and the relatively high quality of their X-ray diffraction patterns makes these viruses uniquely valuable models for the study of the molecular structure of fibrous proteins and

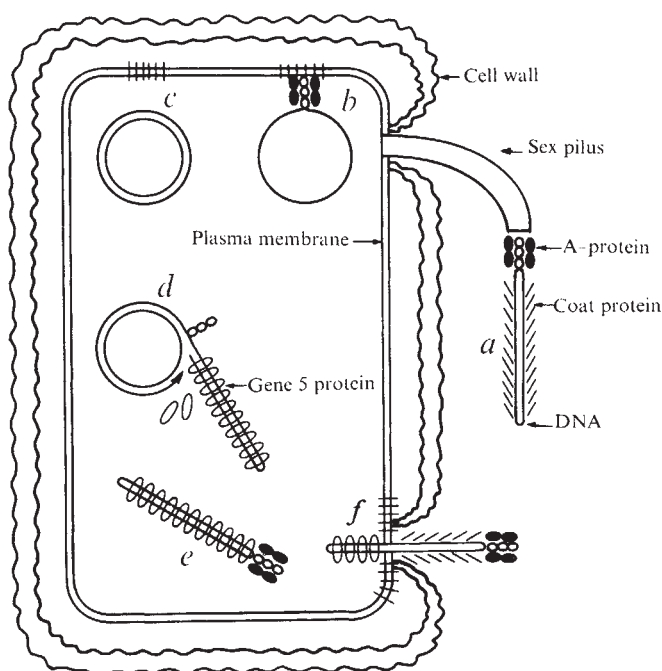


Fig. 1 Schematic illustration of some features of the filamentous bacterial virus life cycle. Successive stages are drawn counter-clockwise around the bacterium, although it is unlikely that all stages would be found at once in any given bacterium. The entire illustration is consistent with current data, but some features are less solidly supported by experiment than others. *a*, The virus attaches to the Gram-negative bacterium by means of a minor coat protein, the A protein (the bacterial attachment site is the sex pilus for some strains² but not others^{28,29}). *b*, The viral DNA and A protein³⁰ enter the bacterium, leaving the major coat protein at the plasma membrane²⁷. *c*, The viral DNA is converted to a duplex form, that replicates to give several hundred progeny duplexes. Viral DNA replication may require the presence of viral coat protein in the membrane³¹, perhaps to create a new site for DNA replication³². *d*, The progeny duplex spins off a single-stranded tail that is coated with the protein product of viral gene 5. *e*, The DNA is closed to give a circular DNA molecule in a linear DNA-gene 5 protein complex³³. *f*, The viral DNA passes out through the bacterial envelope. The gene 5 protein is displaced from the DNA by coat protein that had previously been deposited at the plasma membrane²⁵. The completed virion is released from the bacterium without lysing or otherwise killing the bacterium³⁴. Inhibition of viral assembly in contrast does kill the host in a process involving the gene 5 protein³⁵. More complete citations of the earlier literature are given in ref. 2.

nucleoproteins. In addition, their unusual life cycle (Fig. 1) makes them interesting systems for the study of dynamic molecular interactions such as the displacement of one protein by another from a nucleoprotein complex, and the transport of macromolecules across membranes.

Structures of these viruses

Concentrated gels of filamentous bacterial virus can be oriented in fibres for X-ray diffraction studies³. Two classes of diffraction pattern, differing in detail, were found during a survey of various strains carried out in this laboratory^{4,5}. The class I pattern is given by the fd, f1, M13, If1, and IKe strains⁴; the class II pattern is given by the Pf1 and Xf strains⁵. The class II pattern is simpler to interpret, and has therefore been analysed in more detail⁶. The position of diffracted intensity on the pattern indicates⁶ that the protein subunits in the virus are arranged on a helix of pitch ~ 15 Å, with 4.4 subunits in one pitch length of the helix (Fig. 2). The relative magnitudes of diffracted intensity indicate that the diffracting material at each surface lattice point is elongated into a rod that makes an angle of about 20° with the helix axis, and also tilts from large to small radius in the virus. The orientation determined for the rods of electron density is such that rods originating on one turn of the helix interdigitate between rods originating on the next turn.

Spectroscopic measurements^{7,8} show that the α -helix content

of the coat protein approaches 100%. We have therefore assumed that the rods of electron density found from qualitative analysis of the X-ray pattern can be considered as single rods of α -helical protein measuring 10 Å by 70 Å, the dimensions of an α helix with molecular weight 5,000, and have used model-building techniques for further analysis of the structure. The α helix was represented in initial models by a row of points distributed on a curve 70 Å long. The Fourier transform⁹ of this repeat unit in the virus helix was calculated for various orientations and curvatures of the row, and compared with the class II diffraction pattern. The results of many trial calculations confirmed the conclusions about orientation of rods of electron density that were derived from direct analysis of the diffraction pattern. For the next stage in our model building, the row of points was replaced by a 70 Å polyaniline α helix. We generated the co-ordinates of atoms in this α helix by extending Crick's equation for the coordinates of a coiled coil⁹ to allow for a change in radius of the α helix in the major helix frame. Scattering factors of atoms were modified as described by Wilkins and coworkers¹⁰ to take into account the background electron density due to water and low density protein sidechains¹¹. The contribution of the DNA to the calculated diffraction was ignored at this stage.

Both intrachain and interchain stereochemistry were included as further constraints on the model. The intrachain stereochemistry of the α -helix rod was refined by fitting a stereochemically perfect polypeptide chain to the rough guide coordinates generated for the curved α helix, using the computer program developed by Diamond¹². The Ramachandran angles were $110^\circ < \phi < 133^\circ$ and $123^\circ < \psi < 149^\circ$ over the length of the refined α helix, and internal hydrogen bond lengths of the refined structure were also within the permissible range for α helix¹³. The interchain stereochemistry (the distance between

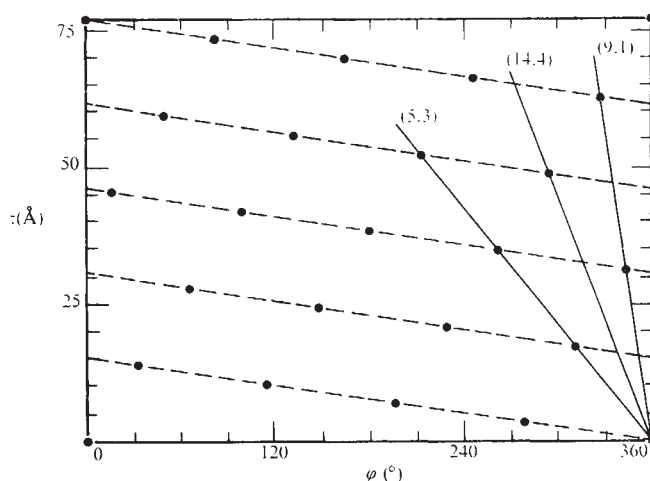


Fig. 2 Surface lattice of the class II helix. The lattice shows the 22 equivalent points in a 77 Å section of the virus helix, projected on to a cylindrical surface coaxial with the virus, which is then opened out flat and viewed from the outside of the helix. The dashed line shows the ~ 15 Å pitch of the virus helix. There are 22 units in 5 turns or 4.4 units per turn⁶. These helix parameters define the order of the Bessel functions predicted on each layer line of the helix diffraction pattern. In particular, for layer lines $l = 1, 2$ and 3 , the lowest order Bessel functions are those with order $|n| = 9, 4$ and 5 , respectively. The sign associated with $|n| = 5$ and 9 is opposite to that associated with $|n| = 4$. The sense of the helices with (n, l) indices $(5, 3)$ and $(9, 1)$ must be the same as that of the ~ 15 Å virus helix (they are shown as left-handed in this figure), but the absolute sense is not defined by the X-ray data. The fact that intensity is stronger on $l = 1$ and 3 than on $l = 2$ indicates that rods of electron density have the same sense as the ~ 15 Å helix. These rods of electron density roughly follow the $(14, 4)$ helix. The maxima of intensity on both $l = 1$ and $l = 3$ are observed at 0.1 Å^{-1} along the layer lines, even though the predicted Bessel function orders are quite different for these two layer lines. This indicates that the rods of electron density tilt from large to small radius as well as slewing around the virus⁶.

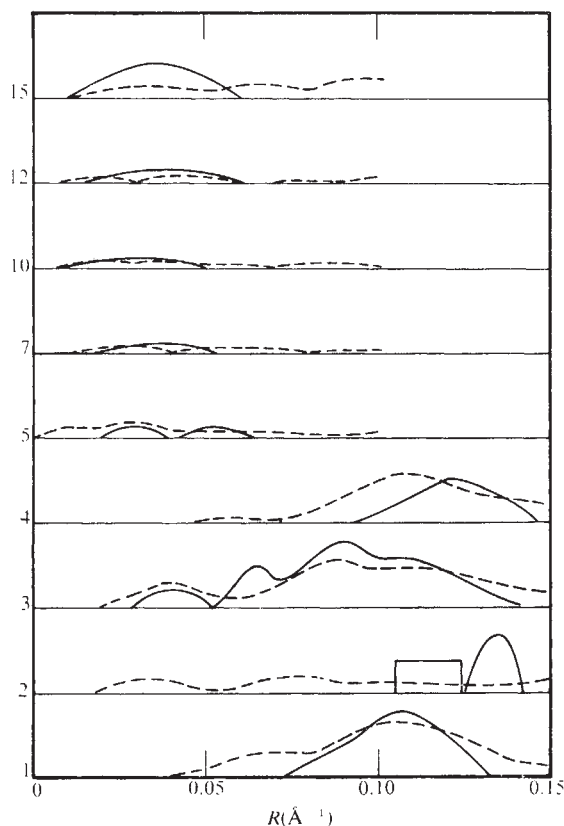


Fig. 3 Comparison of the calculated Fourier transform of the class II protein model with the observed diffraction amplitudes. Continuous diffraction on non-equatorial layer lines was measured^{13,15} on a diffraction pattern of Pf1 taken at 98% relative humidity by Dr R. L. Wiseman⁵ (film No. 2036). The cylindrically averaged Fourier transform was calculated for a repeat unit consisting of a 46-residue stretch of polyaniline α helix the axis of which roughly follows a segment of a conchospiral with $\alpha = -21^\circ$ and $\gamma = 11.5^\circ$ between 14 Å and 26 Å radius. This repeat unit was placed in a left-handed helix having 22 units in 5 turns in an axial repeat $c = 77$ Å. Over most of the region of close contact between neighbouring α helices, distances between the α -helix axes were 10 ± 1 Å and crossing angles between the axes were $9-12^\circ$. Observed amplitudes are solid lines; calculated amplitudes are dashed lines. The rectangular box on $l = 2$ represents integrated intensity for which the shape could not be determined. Analysis of the equator is discussed in ref. 15.

neighbouring α helices and the angle at which the axes of neighbouring helices cross each other) was such that sidechains on one α helix could fit into the space between sidechains on its neighbours in knobs-into-holes packing⁹. Only α helices whose axes follow a left-handed helical path can form satisfactory knobs-into-holes packing, indicating that the sense of the ~ 15 Å virus helix is also left-handed (Fig. 2).

The resolution of the X-ray data is not sufficient to define the positions of specific sidechains, but reasonable assumptions about the orientation of the protein molecule can be made on general chemical grounds. The acidic N-terminal end of the Pf1 protein was placed at the outer radius of the virus, and the basic C-terminal end at the inner radius, as first suggested for fd⁷. The α helix was then rotated about its own axis so that arginine-44 and lysine-45 were directed inwards, towards the virus core; the acidic residues were directed outwards at the virus surface; and most of the hydrophobic residues were involved in contacts with neighbours¹⁴.

There are narrow limits on the range of models that are consistent with all these stereochemical constraints and yet give calculated transforms similar to the observed. The observed and calculated transforms for the current best model are compared in Fig. 3. The model is illustrated in Figs 4 and 5.

Fourier analysis of the equatorial intensity (which gives information about the electron density projected down the long

axis of the virion) shows that the DNA occupies a central core surrounded by a shell of protein, with virtually no interpenetration of DNA and protein¹⁵. X-ray diffraction and chemical evidence, however, are insufficient for a detailed model of the DNA to be built at this time.

Symmetry and perturbation

An overlapping or shingled arrangement of axially elongated protein subunits as found for filamentous bacterial viruses is a stable and durable design for fibrous proteins. Larger bonding surfaces are available for interaction of subunits than would be available in a comparable structure made of globular subunits. In the minimum energy configuration¹⁶ for such an assembly, there are likely to be regular contacts along the length of neighbouring subunits, resulting in similar bonds at different radii. These bonds will be related by symmetry elements that operate not just in the surface lattice of the helix, but in three dimensions.

For the conformation derived experimentally for class II coat protein subunits, the α -helix axis roughly follows a segment of a conchospiral, a space curve with many interesting symmetry properties^{17,18}. The conchospiral is given by the equations

$$\begin{aligned} r &= a \exp(\phi \cot \alpha \sin \gamma) \\ z &= c \exp(\phi \cot \alpha \sin \gamma) \\ \tan \gamma &= a/c, \end{aligned}$$

where r , ϕ , z are cylindrical polar coordinates, α is the constant angle that the tangent to the curve makes with the z axis, and γ is the half-angle of the cone on which the conchospiral lies. This curve is the most symmetrical space curve aside from the circular helix. The curvature and torsion of the curve change continuously in a regular way as one progresses along the curve, giving regular internal symmetry. The segments of conchospiral in the filamentous virus helix are located so that each segment is roughly centred along its length between its nearest neighbours. There are two main kinds of intermolecular contacts: from any protein molecule to the proteins originating five and nine units, respectively, down the ~ 15 Å virus helix (Fig. 5). In the class II structure these contacts are sufficiently similar so that each protein in the helix has essentially equivalent environments in both directions, although a faint meridional reflection on the ~ 15 Å layer line indicates a slight perturbation repeating periodically about once per turn of the ~ 15 Å virus helix. The corresponding meridional reflection on class I patterns, and by implication the associated perturbation, is much stronger⁴. Contacts between the class I α helices that correspond to the 0 to -5 class II contacts in Fig. 5 are regular, but contacts corresponding to 0 to -9 are irregular, with a perturbation that repeats about every five units. This perturbed configuration presumably optimises local contacts and is therefore more stable than any unperturbed configuration that could be formed by the class I subunits.

Three principles for the design of linear assemblies of proteins are suggested by the detailed studies of filamentous viruses. (1) Linear assemblies will often be composed of axially elongated overlapping subunits. (2) Interdigitation of elongated subunits in neighbouring turns of the helical array will be facilitated if there are (roughly) an odd number of units in two turns of the helix. (3) Contacts between interdigitating neighbours will often be improved by a perturbation away from the ideal helical positions, repeating once every turn of the helix, and giving rise to a set of meridional reflections at orders of a periodicity that corresponds to the pitch of the helix rather than to the subunit spacing. Overlapping arrangements of elongated subunits have been proposed for myosin filaments in muscle¹⁹⁻²¹. Sharp meridional reflections have been observed on diffraction patterns of many fibrous proteins¹³, and in some cases have been interpreted in terms of perturbation. Some linear assemblies,

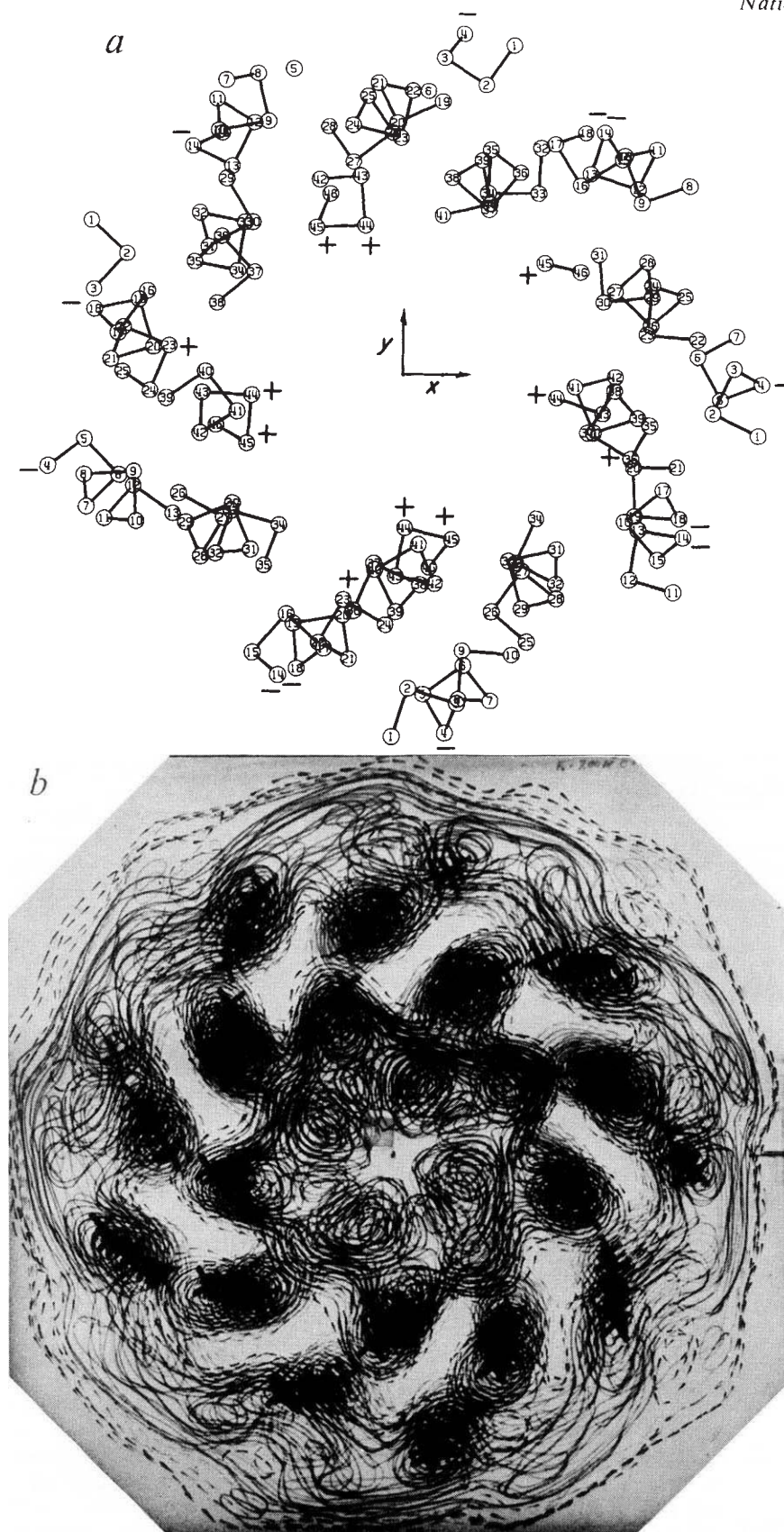


Fig. 4 Axial section of the class II model. A 14 Å slab through one virion, equivalent to four helix asymmetric units, is shown for convenience in visualising the packing of neighbours. *a*, Ball-and-stick representation of the α -carbon atoms, projected on to a plane normal to the axis of the virion. Numbers indicate residues. Several segments of different α helices overlap, but can be distinguished by discontinuities in the sequence of numbers. The basic residues (20, 44 and 45) and acidic residues (4, 14 and 18) are indicated by + and -, respectively. The box measures 30 Å on a side. *b*, Electron density in a corresponding slab, calculated⁶ using³⁸ the observed amplitudes and the phases of the protein model shown in Fig. 3. For the equator, phases were taken from Fig. 7*b* of ref. 15, and amplitudes from continuous transform of a non-crystalline Pf1 fibre at 100% relative humidity, measured and corrected for residual crystalline reflections as described in ref. 15. Crosses indicate the positions of axes of α helices in the model. Zero contours are dashed and may enclose negative density. Although DNA was not included in the phasing, it is expected that phases calculated on the basis of the protein model alone will be approximately correct, and may reveal structure not included in the model, such as possible phosphate peaks in the central DNA region.

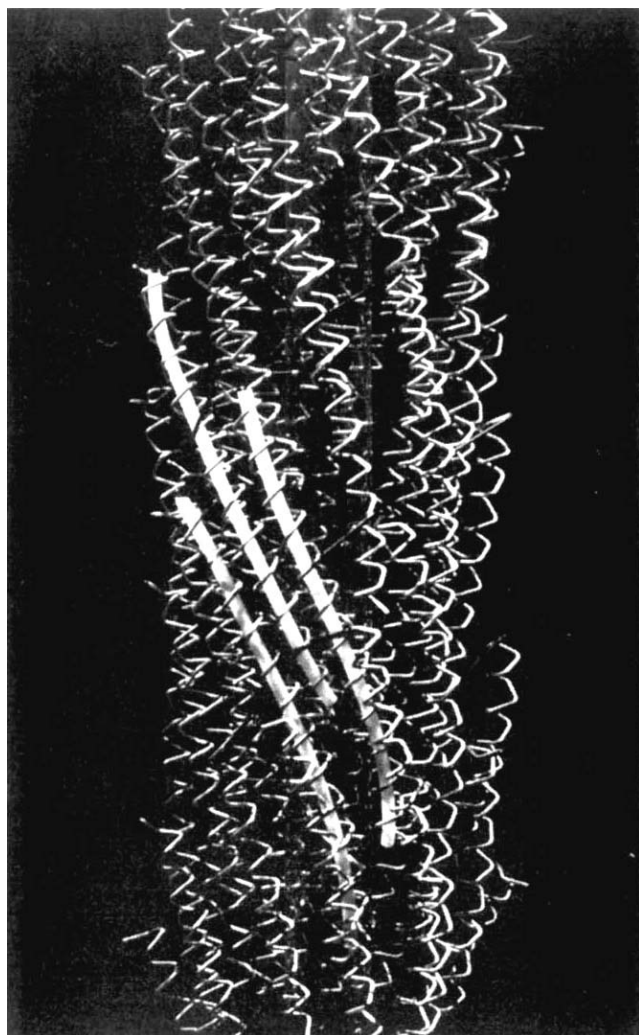


Fig. 5 Model of a 154 Å slab of the protein coat of the virus. The long axis of the virion is vertical. The protein subunits are represented by bent rod following the path from one α carbon to the next. Three neighbouring α helices are picked out by lengths of tubing: they are shown in the same view as Fig. 3 of ref. 14. The right-most α helix originates five units down the ~ 15 Å helix with respect to the central α helix, and the left-most α helix originates nine units down. Note the gentle curve of the α helices, and the interdigitation of neighbouring helices. DNA would occupy the central cylindrical cavity in the protein shell. The outer diameter of the lucite support rod is 6 Å in the model.

such as microtubules or bacterial flagella that have been assumed to be composed of globular subunits²²⁻²⁴ should perhaps be re-examined in the light of these principles.

Assembly

With the molecular structure of the virion in hand, we can discuss specific hypotheses about viral assembly. We assume the model of assembly illustrated in Fig. 1, and restrict ourselves to the steady-stage of assembly, after the forward end of the virion has passed across the membrane. The viral coat protein is a single rod of α helix with a hydrophobic central portion and hydrophilic ends. The protein could be excreted directly into the lipid bilayer²⁵ during synthesis²⁶, with its N-terminal end outwards and its long axis normal to the plane of the bilayer². The DNA, stripped of the gene 5 protein would, form a condensation centre for the coat protein previously stored in the bilayer. As the protein assembled on to the DNA, its hydrophobic areas would become masked, so that the protein would become less soluble in lipid than in water and therefore

would leave the lipid for the water, taking associated DNA with it. The process would be akin to crystal growth where the growing point of the crystal is fixed in space so that the crystal must move away from the growing point. The sense of the process (the virion moves out of rather than into the bacterium) would depend on the details of the configurational change in the coat protein upon assembly. A similar process in reverse could drive the dissolution of protein off the virion into the lipid bilayer²⁷ and advance the DNA into the bacterium during infection. This model—that assembly of hydrophobic proteins within a lipid bilayer causes changes in the properties of the completed assembly so that it becomes more soluble than the monomer in an aqueous phase and thus moves out of the bilayer—could apply in general to transport of oligomeric or polymeric proteins through membranes.

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letters to nature

Measurement of circular polarisation in the Crab Nebula at 1,415 MHz

RECENTLY Rees and Gunn¹ suggested that the 30-Hz wave power probably emerging from the Crab Nebula Pulsar NP0532 does not propagate further than a small region around the pulsar with a radius of about 10% of the nebular radius. Beyond this, in the main body of the nebula, the magnetic field is built up in toroidal form to equipartition strength so that the continuum emission from the bulk of the nebula is entirely synchrotron radiation. The axis of this toroid is aligned with the rotational axis of the pulsar which is assumed to be lying in the plane of the sky along the major axis of the nebula (NW/SE). A test of this proposal is the measurement of a characteristic sign change in the distribution of Faraday rotation or circular polarisation over the nebula. If the nebula is divided into quadrants along its major and minor axes, then adjacent quadrants should have the opposite sign and opposite quadrants the same sign. Since any measured Faraday rotation does not necessarily arise within the body of the nebula, the critical test becomes a detection of the characteristic sign distribution in the small amount of circular polarisation ($\sim 0.1\%$) expected at radio frequencies from ordinary synchrotron radiation². The theoretical frequency dependence of $\nu^{-1/2}$ for the fraction of circular polarisation would then explain the null result of Landstreet and Angel³ (see also ref. 4) at optical frequencies. Here I report a measurement of the distribution of circular polarisation (Stokes parameter V) in the Crab Nebula at 1,415 MHz obtained during a series of high accuracy full polarisation measurements with the Westerbork Synthesis Radio Telescope in November 1971.

A brief description of the normal method of polarisation measurement with the Synthesis Telescope can be found in ref. 5. In our case, this was modified by having, in each of the 20 simultaneous interferometers, the crossed dipoles in both telescopes parallel rather than at the usual 45 degrees to each other. The corrections for instrumental polarisation of the order of 1% of the total intensity were determined by observing 3C48 for 2×12 h and assuming that it was totally unpolarised at 1,415 MHz. It has been shown by Conway *et al.*⁶ ($V = +0.015 \pm 0.010\%$ at 1,415 MHz) and by Berge and Seielstad⁷ ($V = -0.024 \pm 0.024\%$ at 1,420 MHz) that this assumption is accurate for the V component with which I am concerned here. The final accuracy of the measurement of the distribution of circular polarisation in the Crab Nebula was then approximately 0.02% of the total intensity.

The great radio flux of the nebula overloaded all interferometer baselines shorter than 300 M and these had to be eliminated from the observation. Thus, the circular polarisation map is mainly sensitive to the smaller scale features in the source and not to the broader extended structure.

The resulting map of the distribution of circular polarisation in the Crab Nebula is presented in Fig. 1. The vertical and horizontal scales refer to the position of the pulsar (the cross). The r.m.s. noise error in the map is approximately one contour. Because of the missing short interferometer spacings, the area of the synthesised beam is not well defined and a conversion of the brightness contours to units of temperature will not be attempted. Even though V can assume both positive and

negative values, the negative feature to the west of the pulsar cannot, in this case, be distinguished from the effect of the missing short spacings. Also, since its magnitude is only about twice the r.m.s. noise error in the map, I do not consider it to be a real negative component of circular polarisation. Similarly, although feature A on the map cannot be generated by the effect of missing short spacings, it is only twice the r.m.s. noise error and thus of marginal significance.

For comparison with the distribution of total intensity, Fig. 2 shows the total intensity map from Duin and van der Laan⁸ obtained with the Westerbork Synthesis Telescope in a special detuned observing configuration which avoided the saturation problems discussed above. The frequency of observation and the resolution are the same as in Fig. 1 and a direct comparison of the maps can be made. From this it is possible to compute the circular polarisation for the four lettered areas as 0.04% (A), 0.05% (B), 0.06% (C) and 0.05% (D). These values can only be rough estimates due to the possibility of an undetected larger scale background in the source. They are, however, consistent with the amount of circular polarisation

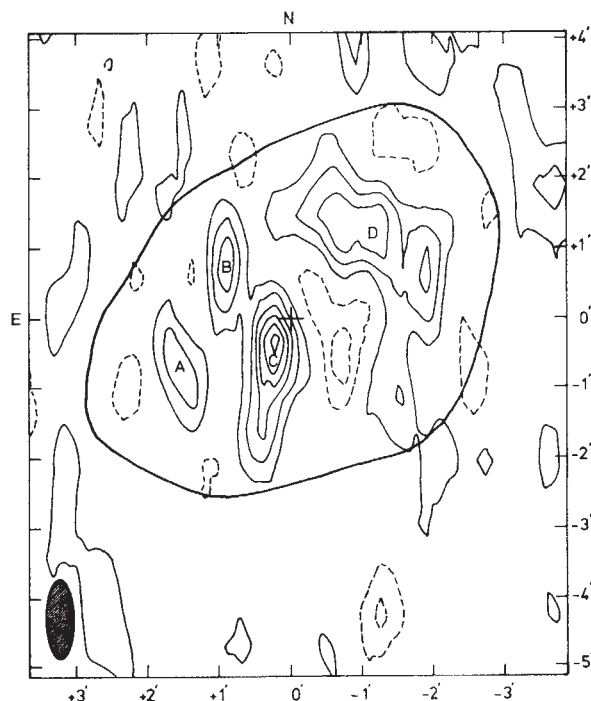


Fig. 1 Contours of the circular polarisation (Stokes parameter V) for the Crab Nebula at 1,415 MHz. The contour interval is 2.5 mJy per synthesised beam area. Positive contours are solid and represent right-hand circular polarisation (IRE definition). Negative contours are dashed and no zero contour is plotted. The heavy outside contour (taken from Duin and van der Laan)⁸ is where the total intensity has 10% of its peak value. The cross marks the position of NP0532 (α (1950.0) = 05 h 31 min 31.46 s; δ (1950.0) = $21^\circ 58' 54''.8$). The half-power synthesised beam width is shown in the lower left corner.

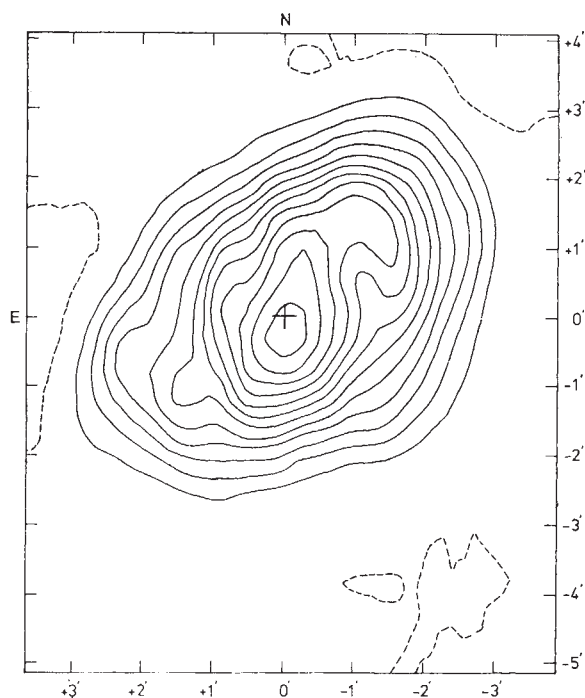


Fig. 2 Contours of the total intensity (Stokes parameter I) at 1,415 MHz from Duin and van der Laan⁸. The contour interval is 2 Jy per synthesised beam area and the peak brightness is 25 Jy per synthesised beam area. The zero contour is plotted dashed. The cross marks the position of NP0532. The resolution and horizontal and vertical scales are the same as in Fig. 1.

expected from normal synchrotron radiation in a static magnetic field of 10^{-3} to 10^{-4} gauss.

The major feature of Fig. 1 is that whenever circular polarisation is detected, its sign is positive, indicating an average line-of-sight component of the magnetic field directed towards us. There is no indication of the sign reversal between adjacent quadrants expected from a toroidal structure.

The simplest magnetic field structure consistent with the present measurements is that of a relatively uniform field of 10^{-3} to 10^{-4} gauss directed with its line-of-sight component towards us. This form and strength is then quite similar to that suggested by Weiler and Seielstad⁹ from synthesis measurements of the linear polarisation and Faraday rotation at radio wavelengths. An effective mechanism for maintaining and amplifying such a field during the lifetime of the nebula is, however, still needed.

An independent confirmation of the circular polarisation in the Crab Nebula would be extremely useful. Thus, it should be noted that the presence of only positive values of V in the synthesis map implies that the integrated radiation should have a detectable amount of circular polarisation at radio frequencies. This could be of the order of 0.05–0.1% at 1,415 MHz and theoretically more at lower frequencies. There is only one previous measurement of the fraction of circular polarisation in the integrated radiation of the Crab Nebula at radio wavelengths which exceeds 0.1% in accuracy. Seielstad and Weiler⁷ found $+0.13 \pm 0.09\%$ circular polarisation at 1,418 MHz from measurements with the Owens Valley interferometer. Although this is insufficiently larger than the errors to be trusted, it is interesting that it agrees in sign with the present measurements.

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K. W. WEILER

*Netherlands Foundation for Radio Astronomy and
Kapteyn Astronomical Institute,
University of Groningen,
Groningen, The Netherlands*

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Nucleosynthesis and matter–antimatter cosmologies

If light elements are of cosmological origin, then a given cosmological model should be able to reproduce the observed abundances¹. Symmetric matter–antimatter cosmologies^{2–4} do not stand up to this test too well, and encounter problems whose basis is the annihilation process itself.

Steigman⁵ has emphasised that there is an important loss of neutrons at temperatures of the order of 1 MeV. (The matter–antimatter separation is assumed⁴ to have taken place at temperatures of the order of 350 MeV.)

Protons are essentially ‘frozen’ within a matter region by electrostatic interactions, and do not diffuse towards the matter–antimatter interface; this, however, is not true of neutrons, which are affected only by the interactions of their magnetic moment with that of the electrons (for which the cross section is very low) and by elastic collisions with protons. When the temperature has dropped below 1 MeV, weak interactions can no longer maintain an equilibrium between the protons and neutrons, and the neutron abundance in a region within a diffusion length of the interface ($\sim \sqrt{Dt}$), where D is the neutron diffusion coefficient and t the time) will drop as a consequence of the annihilation which occurs there.

It follows that the neutron–proton ratio, on which nucleosynthesis depends, is essentially a function of the ratio of the neutron diffusion length to the mean size of a matter region. Now, according to Omnès⁶, the size of a matter region turns out to be of the order of a neutron diffusion length: the neutrons must therefore all escape and be annihilated. Under these conditions primordial nucleosynthesis cannot take place.

Nevertheless, this estimate for the typical size of a matter region is not necessarily the only possible one, since there is some considerable uncertainty about the physical processes which occur at temperatures above 1 MeV; one might therefore try to fix empirically the ‘size of the emulsion’ (the somewhat misleading term often used to denote the size of a matter or antimatter region) by requiring that the symmetric model give the observed abundances.

To do this, we have to modify the usual evolution equation for the neutrons⁶, since the annihilation follows diffusion. If we suppose that all neutrons which reach the interface are annihilated, the evolution equation can be written as:

$$dn/dt = \lambda p - \hat{\lambda} n - (Dt/4\pi)^{1/2} n/Lt \quad (1)$$

using the notations of ref. 6, D being the neutron diffusion coefficient and L the emulsion size (L is a function of temperature). The proton evolution equation is written in the usual way, since the diffusion is negligible:

$$dp/dt = \hat{\lambda} n - \lambda p \quad (2)$$

If we suppose that L varies with temperature as $L = L_1 T^{-1}_{\text{MeV}}$ cm, we can integrate equations (1) and (2) to obtain a lower limit for the emulsion size at $T=1$ MeV: that is, $L_1 \gtrsim 3 \times 10^6$ cm. The calculations have been done using various other equations for L (simple power laws, a sequence of different power laws joined piecewise), and it turns out that the smallest emulsion size is obtained by taking account only of expansion. This is the smallest emulsion size for which annihilation does not affect the relative neutron abundance: therefore, at the end of nucleosynthesis ($T \sim 0.1$ MeV), the light elements will have their usual¹ abundances.

At this epoch, the Universe is a matter-antimatter emulsion consisting for the most part of protons and helium nuclei together with the corresponding antielements. Although nucleosynthesis has finished, the annihilation processes themselves continue: an antiproton reaching the interface can annihilate against a proton or one of the nucleons within a helium nucleus, producing in the latter case a deuteron together with a number of neutrons. The deuterons produced in this kind of reaction will have a large kinetic energy, will leave the annihilation zone and, being charged, will be slowed down rapidly in the matter region. We shall call this 'direct deuterium'. It can be shown that all the neutrons will also be thermalised by elastic collisions with protons and helium nuclei; note, however, that this process is somewhat slower. As soon as they are sufficiently slow, they are captured by the protons to create more deuterium—this will be referred to as 'indirect deuterium'. The capture cross section rises very rapidly at low energies.

The annihilation rate per unit surface depends only on the nucleon flux at the interface, and so is independent of the emulsion size. Therefore, the annihilation rate per unit volume is inversely proportional to L : the smaller L , the larger the number of annihilations $\bar{p}-\alpha$ and the larger the amount of deuterium produced. Now, we must not at this stage produce too much deuterium (subsequent stellar destruction cannot lower the amount by an order of magnitude)—deuterium formation after the end of nucleosynthesis introduces therefore a new constraint on the emulsion size.

To calculate the amount of deuterium produced by this process, we have to know the branching ratios of the reactions $\bar{p}-\alpha \rightarrow d + \text{anything}$ and $\bar{p}-\alpha \rightarrow n + \text{anything}$. Since very little experimental and theoretical information is available, the following very rough model has been used. We suppose that the low energy ($E_{\text{kin}} \leq 1$ MeV) antiproton annihilates against the first nucleon which it meets in the ^4He nucleus, and produces an average of 5–6 pions. Experiment^{7–10} suggests that, on average, only one of these pions interacts with the remaining nucleons, which are still sufficiently close together to be treated as a ^3He or ^3H nucleus. The reaction $\pi-^3\text{He}$ (ref. 11) has been studied experimentally for pions at rest, and so the number of deuterons produced per interaction (~ 0.25) is probably overestimated and the number of neutrons (~ 1.75) underestimated. There could be ~ 7 times as much indirect deuterium produced as direct if each neutron were captured: detailed calculations¹², which take into account the path lengths of the various different particles, show that to a first approximation we may in fact neglect the direct deuterium.

An appreciable quantity of deuterium will be produced only if the capture process $p+n \rightarrow d + \gamma$ be more important than the photodissociation $d + \gamma \rightarrow p + n$, that is if the temperature be below 0.07 MeV. We shall denote the time at which this temperature is reached by t_i . This production stops when the temperature is so low that the neutron decay has become more important than capture: this occurs at the time t_f . The deuterium abundance is therefore calculated for the interval $t_i - t_f$:

$$d/p = \int_{t_i}^{t_f} (vL^2 B) \times \frac{1.75}{2} dt/pL^3$$

where d and p are the number densities of deuterium and protons respectively, v is the $\bar{p}-p$ annihilation rate (it can easily be shown that $B \sim \alpha < \sigma v >_{\bar{p}\alpha}/p < \sigma v >_{\bar{p}p}$, where α is the number density of helium, the other quantities having their usual meaning¹³), $L = L_1 T^{-1}_{\text{MeV}}$ is the emulsion size. Note that we have introduced a factor $\frac{1}{2}$ because, on average, one half of the products of a $\bar{p}-\alpha$ annihilation pass into a matter region, and one half into an antimatter region.

In order that this process does not produce more deuterium than is observed¹⁴ $d/p \lesssim 10^{-5}$. The emulsion size at a temperature of 1 MeV is therefore given by $L_1 \geq 1.5 \times 10^8 (\Omega h^2)^{1/3}$ cm, where Ω is the density parameter and $h' = H_0/50$ (H_0 is the Hubble constant in $\text{km s}^{-1} \text{Mpc}^{-1}$).

During the radiative era, the nucleon number density rises by a factor of $\sim 10^3$ as a consequence of annihilation; any subsequent annihilation will therefore affect the element abundances very little.

So the amount of helium and deuterium produced as a consequence of nucleosynthesis can be made to agree with observed abundances only if the emulsion size at 1 MeV exceeds the neutron diffusion length by a factor of about 10^3 (at this epoch, $\sqrt{Dt} \sim 10^{4.5}$ cm).

Nevertheless, it would at this stage be unwise to discuss the future of symmetric cosmologies. Our present state of knowledge does not allow us to calculate the emulsion size in any definitive way.

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F. COMBES
O. FASSI-FEHRI
B. LEROY

Observatoire de Paris-Meudon,
Département d'Astrophysique Fondamentale,
92190-Meudon, France

Received September 20, 1974.

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Of fundamental electrodynamics and astrophysics

MAXWELL'S equations are well established for phenomena on laboratory and terrestrial scales, but there are other electrodynamic theories, as yet indistinguishable on such scales from Maxwell's theory, that have significantly different consequences for phenomena on an astronomical scale. For example, extensive magnetic fields are possible in the Universe¹ because of the general absence of magnetic monopoles. Parker¹ considered the abundance of magnetic monopoles that would neutralise or dissipate the magnetic fields of the Earth, Sun and the Galaxy, and deduced for the number density of free magnetic monopoles upper limits that are more stringent than those deduced from laboratory and terrestrial data.

Another example of electromagnetic equations that might be relevant to the description of astrophysical phenomena are the Proca equations, which may be interpreted in terms of a non-zero photon rest mass. A consequence of the Proca equations is that static electric and magnetic fields in free space decay exponentially with distance from the source: for phenomena on scales comparable with or exceeding the characteristic length for decay, the description provided by the Proca equations departs from that provided by the Maxwell equations. In static space-time the Proca equations, like the Maxwell equations, predict that electromagnetic waves propagate with fixed frequency; contrary to what has been claimed^{2,3}, the Proca equations do not form a basis for the 'tired-light' interpretation of the cosmological redshift.

Laboratory and terrestrial experiments have determined⁴ that the photon rest mass, m , is less than about 3×10^{-48} g; astrophysical considerations have led to an improvement by about four and a half orders of magnitude⁵. Here, we obtain an upper limit on m from consideration of the Proca equations and the mass of the Galaxy.

The Proca equations show that $(\mathbf{E}^2 + \mathbf{H}^2 + \mu^2\phi^2 + \mu^2\mathbf{A}^2)/8\pi$ can be interpreted as the energy density of the electromagnetic field⁴; $\mathbf{E}$ and $\mathbf{H}$ are the electric and magnetic fields, ϕ and $\mathbf{A}$ are the scalar and vector potentials and $\mu^{-1} = h/2\pi mc$, where μ^{-1} is the reduced Compton wavelength of the photon, h is Planck's constant and c is the relativistic limiting speed. If ρ denotes an upper limit on the mean mass density of matter and energy in all forms that could exist in a region of space in which a magnetic field is observed, the $\mu^2\mathbf{A}^2/8\pi c^2 \lesssim \rho$. The relationship $\mathbf{H} = \nabla \times \mathbf{A}$ shows that $|\mathbf{A}| \sim |\mathbf{H}| L$ where, for an approximately uniform magnetic field, L is of the order of the smallest dimension. Thus, the above inequality leads to

$$\mu^2 \lesssim 8\pi\rho c^2/\mathbf{H}^2 L^2 \quad (1)$$

The Galactic magnetic field in the vicinity of the Sun has been observed⁶ to have a strength of about 2×10^{-6} gauss and is approximately uniform over a distance of at least 300 pc, so that in the Galactic disk L may be estimated as greater than about 10^{20} cm. The masses of galaxies may be estimated from their rates of differential rotation and from the orbital velocities of binary systems; it has been deduced⁷ that for many galaxies the mass exceeds the total mass of known stars by an order of magnitude. The mass of the Galaxy is often quoted⁸ at about $10^{11} M_{\odot}$ so that $10^{12} M_{\odot}$ is a conservative upper limit on the mass of the Galactic disk. Thus, using the dimensions given by Allen⁸, $\rho \lesssim 10^{-21}$ g cm⁻³ for the Galactic disk. Assuming that the interstellar magnetic field in the vicinity of the Sun is typical of magnetic fields of the Galactic disk, equation (1), with $|\mathbf{H}| \sim 2 \times 10^{-6}$ gauss, $L \gtrsim 10^{20}$ cm and $\rho \lesssim 10^{-21}$ g cm⁻³, leads to $\mu \lesssim 3 \times 10^{-14}$ cm⁻¹, corresponding to $m \lesssim 10^{-51}$ g. This limit is not far from the best available upper limit⁵ of 10^{-55} g.

In interstellar space the energy densities resulting from radiation from stars, turbulent gas motions, universal background radiation and cosmic rays are all of a similar order of magnitude to the Maxwellian energy density, $\mathbf{H}^2/8\pi$, associated with the

magnetic field⁸; if $m \sim 10^{-52}$ g, then the energy density $\mu^2\mathbf{A}^2/8\pi$ exceeds these energy densities by 11 or more orders of magnitude. It may well be that the upper limit of 10^{-52} g could be improved by consideration of the possible sources of the energy density, $\mu^2\mathbf{A}^2/8\pi$, associated with large scale magnetic fields and of instabilities that may result from this energy density. Conversely, it is worth bearing in mind that large energy densities associated with large scale magnetic fields and a non-zero photon rest mass could be significant in some astrophysical processes.

J. C. BYRNE*

R. R. BURMAN

Department of Physics,
University of Western Australia,
Nedlands, Western Australia 6009

Received September 20, 1974.

*Present address: Department of Physics, Imperial College of Science and Technology, London, UK.

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Oblique rotators in binary systems

RADIO pulsar PSR1913+16, which has a period of about 59 ms, has been identified as a member of a binary system¹. The X-ray 'oscillars' HerX-1 and CenX-3, which are also members of binary systems, are not radio pulsars. (I use the word 'oscillars' to distinguish these objects from pulsars, which maintain their periodicity much more accurately). These facts, together with the 34-d periodicity^{2,3} of HerX-1 and the lack of this periodicity in CenX-3, can be explained by using the rotating neutron star hypothesis for the compact object in all of these systems. As is common in pulsar theories, I assume that rotating neutron stars are oblique rotators, that they have magnetic fields and that the magnetic axes are not along the axes of rotation.

An oblique rotator, with a magnetic field, B , a period of rotation, P , and radius, R , gives out low frequency radiation with energy⁴:

$$E \simeq 10^{-28} B^2 R^6 / P^4 \text{ erg s}^{-1}$$

Using $B \sim 10^{12}$ gauss, $R \sim 10^6$ cm and a period, P , of 59 ms, PSR1913+16 gives out about 5×10^{37} erg s⁻¹ of low frequency radiation. This is quite close to the Eddington limit and can equal it with only a slight variation of B or R . Radiation of this intensity opens the equipotential lines near the first Lagrangian point⁵, and accretion of matter from the binary companion is thus prevented. The radio emission, which is attributed to a polar phenomenon⁶ can, therefore, occur without interference from accreting matter. The lack of accretion prevents PSR1913+16 from becoming an X-ray oscillator like HerX-1 or CenX-3. On the other hand, PSR1913+16 could be an X-ray pulsar like NP0532, but with considerably smaller flux.

HerX-1 has an X-ray periodicity of 1.24 s and if this is attributable to the rotation period of the neutron star, the low frequency radiation emitted is about 5×10^{31} ergs s⁻¹. CenX-3 has a period of 4.8 s and gives out about 2×10^{29} erg s⁻¹.

These values for energy emission are considerably smaller than the Eddington limit and so matter could accrete on to the neutron star. If the radio emission process is to continue, that matter must absorb the radio radiation by the free-free process. This can be verified by considering the rate of accretion implied by the X-ray emission of HerX-1 and CenX-3. Thus, it can be seen why HerX-1 and CenX-3 are not radio pulsars.

The low frequency radiation emitted by HerX-1, though not large enough to prevent mass accretion, still regulates it in an interesting manner. Suppose that the density of matter at a distance r from a neutron star of mass, m , ($\sim 1M_{\odot}$) is ρ . Then, equating the radiation pressure and the gravitational force:

$$GM\rho/r^2 = 5 \times 10^{31}/4\pi r^2 c$$

where G is the gravitational constant and c is the velocity of light. This gives $\rho \simeq 10^{-6} \text{ g cm}^{-3}$, and thus in the case of HerX-1 unless the density is higher than about $10^{-6} \text{ g cm}^{-3}$, accretion cannot occur. The matter accreting from the binary companion piles up at the light cylinder and when the density exceeds the calculated value accretion starts and continues until the density again reaches the critical value. Thus, it could be taking 22 d to accrete enough matter to reach the required density, and 12 d for all the released matter to spiral into the neutron stars; during which time X-ray emission occurs. That would also allow the emission of X rays during the 22-d quiescent period, as has been observed⁷. For when the companion star ejects matter as, for example, in a flare, the density in the reservoir may exceed the required value in less time than the average accretion time and so X-ray emission could occur.

In the case of CenX-3, the emission of low frequency radiation is much smaller than in HerX-1, and only low densities of matter are needed to overcome the radiation pressure. Therefore, accretion can occur all the time.

Another interesting effect on the oblique rotator in a binary system is the oscillation of the magnetosphere of the neutron star. This occurs because of the gravitational force of the companion star on the matter in the magnetosphere, somewhat similar to the tidal effect on Earth. This makes the lines of force, which are assumed to be frozen in, vibrate as the neutron star rotates. By calculation I found that in the case of HerX-1 the fluctuation of the field intensity is 100% at around a few times 10^9 cm . The effect of this fluctuation is to accelerate particles. But the energy involved is only about 10^{32} – $10^{33} \text{ erg s}^{-1}$. A detailed account will be presented elsewhere.

Note added in proof: Margon *et al.*⁸ observed PSR1913+16 for an hour using the Copernicus satellite, for X-ray emission in the region 1–3 Å. They did not find any X-ray flux and give an upper limit to $10^{-10} \text{ erg cm}^{-2} \text{ s}^{-1}$. The non-observation of X-ray flux from PSR1913+16 is consistent with the model suggested above, since the low frequency radiation prevents accretion.

KRISHNA M. V. APPARAO

Tata Institute of Fundamental Research,
Homi Bhabha Road,
Bombay 500 400, India

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Polarisation of atmospheric bremsstrahlung

THE terrestrial atmosphere is bombarded continuously by energetic charged particles of extraterrestrial origin. When a charged particle penetrates deep into the atmosphere, either its direction changes or its speed varies with respect to the local phase velocity of electromagnetic waves. These changes, caused by the dense atmosphere at altitudes of about 90–100 km, generate bremsstrahlung X rays. The flux of atmospheric bremsstrahlung X rays has been measured using balloons^{1–3}, rockets and satellites^{4,5}. Measurement of the atmospheric X-ray flux has become routine in the study of electron flux precipitation into the lower ionosphere^{6,7}. The degree of polarisation of emitted radiation changes rather drastically with the changing energy of precipitating electrons and with the height of precipitation. But no effort has yet been made to measure the polarisation of atmospheric bremsstrahlung. We here show the potential importance of bremsstrahlung X-ray polarisation and its probable role in atmospheric diagnostics.

In the auroral zone electron penetration is almost vertically downwards. Electrons penetrating the dense atmosphere give rise to linearly polarised bremsstrahlung X-ray photons. The degree of linear polarisation is a function of the kinetic energy of scattering electrons, the energy of the emitted photons, the angle of photon emission with respect to the direction of the incident electron, and the atomic number density of atmospheric gas. The classical and quantum mechanical theories of linear polarisation of bremsstrahlung X rays have been fully developed. The success of a diagnostic technique is based primarily on the compatibility between experimental measurements and theoretical computations. But the theoretical computation of the degree of linear polarisation using rigorous quantum mechanical theory, and the detailed nature of target structure have posed a serious problem. This has inhibited the use of the degree of polarisation as a diagnostic measure. Kirkpatrick and Wiedmann⁸ have developed an approximate computational procedure for obtaining the degree of polarisation as a function of the projectile and target parameters. Using that procedure we have computed the height profile of the degree of polarisation of bremsstrahlung X rays in the terrestrial atmosphere and have shown its variation with related parameters.

Bremsstrahlung X rays received at a point in the z - x plane (z being vertical), at an angle θ relative to the incident electron direction can be resolved into two linearly polarised intensity components, $I_{\perp}$ and $I_{\parallel}$. The radiated X ray is assumed to be linearly polarised in the direction of the emitting equivalent

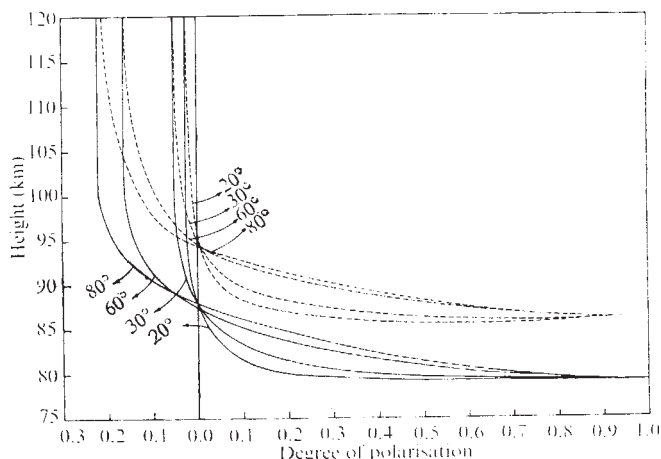


Fig. 1 Variation of the degree of polarisation with height of 20 and 40 keV electrons (dashed and solid lines, respectively) for different angles of photon emission.

dipole. Therefore, $I_{\perp}$ will have only a y component whereas $I_{\parallel}$ will have x and z components. Thus:

$$\begin{aligned} I_{\perp} &= I_y, \\ I_{\parallel} &= I_x \sin^2\theta + I_z \sin^2(90^\circ - \theta) \end{aligned} \quad (1)$$

For an unpolarised electron beam there exists an axial symmetry so that

$$\begin{aligned} I_y &= I_z, \\ \text{and } I_{\parallel} &= I_x \sin^2\theta + I_y \cos^2\theta \end{aligned} \quad (2)$$

From equations (1) and (2) the degree of linear polarisation can be defined as

$$P(\theta) = \frac{(1 - I_y/I_x)}{[(I_y/I_x)(2\csc^2\theta - 1) + 1]} \quad (3)$$

By this definition the degree of polarisation, $P(\theta)$, ranges from $+1$ (complete polarisation perpendicular to the electron-photon plane) to -1 (complete polarisation parallel to that plane).

In view of the difficulties involved in the computation of I_x and I_y using rigorous quantum mechanical theory we have resorted to an approximate method following Kirkpatrick and Wiedmann⁸:

$$I_x = 10^{-50} (Z^2/V) [0.252 + a((v/v_0) - 0.135) + b((v/v_0) - 0.135)^2] \quad (4)$$

and

$$I_y = I_z = 10^{-50} (Z^2/V) [-j + k/(v/v_0 + h)] \quad (5)$$

where v_0 and v are the photon frequencies corresponding to the initial and final energies of the scattering electrons, V is the velocity of electrons in e.s.u. and Z is the atomic number of the scatterers. The constants a, b, h and k are empirical constants defined by Kirkpatrick and Wiedmann⁸.

The height profile of V/Z^2 and v/v_0 have been obtained from detailed computation of the energy degradation of charged particles into the ionosphere^{9,10}. I_x and I_y are functions of the height profiles, V/Z^2 and v/v_0 which give rise to the height profile of $P(\theta)$ (Fig. 1). It is obvious from Fig. 1 that measurement of the degree of polarisation, $P(\theta)$, at a particular height is very sensitive to changes in the energy of incident electrons and to the direction of the emitted photons. The height of the transition of the plane of polarisation from $+$ to $-$ changes with the changing incident energy of the electrons. The height of the transition is, however, independent of the angle of bremsstrahlung photon emission. This variation with height suggests that simultaneous measurements at different heights could allow the prediction of degradation of electron energy and changes in electron flux. The lack of a high efficiency, high resolution X-ray spectrometer has been the main reason for the paucity of experimental measurements of the polarisation of bremsstrahlung radiation produced by low energy electrons. The advent of the Ge(Li) detector has solved this problem and it seems quite feasible that the measurement of polarisation could be used as a diagnostic probe for studying precipitating electron energy and flux.

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R. R. RAUSARIA
R. N. SINGH

Physics Section,
Institute of Technology,
Banaras Hindu University,
Varanasi 221005, India

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Landau damping and cyclotron resonance in uniform magnetoplasma

THE formation of a ledge in the electron distribution functions during Landau damping produces steep gradients at the boundaries of the disturbed region of the distribution function. This makes the pitch angle distribution sufficiently anisotropic to amplify a gyroresonant whistler mode perturbation at the appropriate frequencies.

The system under consideration consists of a homogeneous, collisionless hot plasma in a uniform magnetic field B_0 , a spectrum of electron plasma waves with their propagation vectors k antiparallel to B_0 , and a wide band whistler mode perturbation with k parallel to B_0 . For simplicity, the particle distribution f is taken to be initially independent of pitch angle $\alpha = \tan^{-1}(v_{\perp}/v_{\parallel})$ where v is the particle velocity and the subscripts $\parallel$ and $\perp$ refer to the vector components along and perpendicular to B_0 .

The electron plasma waves would be Landau damped with a growth rate¹ γ_L varying as $\partial f / \partial v_{\parallel}$ at $v_{\parallel} = v_{\text{res}}$, where $v_{\text{res}} = \omega_p/k$ is the Landau resonant velocity, ω_p being the electron plasma frequency. Simultaneously, the electron distribution would evolve quasilinearly in accordance with the equation¹:

$$\partial f / \partial t = (\partial / \partial v_{\parallel}) \{ (4\pi e^2 / m^2 v_{\parallel}) (E_k^2 / 8\pi) \} (\partial f / \partial v_{\parallel}) \quad (1)$$

where e and m are the electron charge and mass respectively and $E_k^2 / 8\pi$ is the energy density of the wave.

The growth rate² γ_c for the whistler mode perturbation, which results from cyclotron resonance with electrons is proportional to $(A(V_R) - B)$ where

$$A(V_R) \propto \left[\int_0^{\infty} v_{\perp} dv_{\perp} \{ v_{\parallel} (\partial f / \partial v_{\perp}) - v_{\perp} (\partial f / \partial v_{\parallel}) \} v_{\perp} / v_{\parallel} \right]_{V_{\parallel} = -V_R}^{V_{\parallel} = V_R} \quad (2)$$

and $B = \omega / (\Omega - \omega)$ with $V_R = (\omega - \Omega) / k$ and $\Omega = eB_0 / mc$.

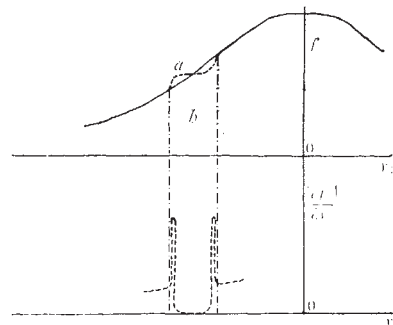


Fig. 1 Upper part: f against $v_{\parallel}$, k is negative. The ledge (a) forms with negative values of $v_{\parallel}$, (b), Disturbed region. Lower part: $\partial f / \partial v_{\parallel}$, at different points of the ledge, against $v_{\parallel}$. The steep rise in f at the boundaries of b gives the peaks in $|\partial f / \partial v_{\parallel}|$.

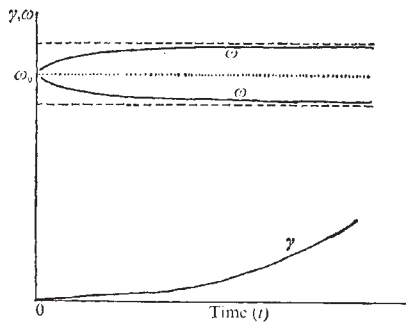


Fig. 2 Behaviour of growing waves with time. Upper part: frequency growing fastest at time t , against t . The curve has two branches, one above and one below the central frequency. Lower part: maximum growth rate at time t , against t .

The cyclotron growth of the perturbation, in turn, affects the pitch angle distribution of the particles, which then evolves in accordance with the equation² (for $\omega \ll \Omega$):

$$\frac{\partial f(v, \alpha, t)}{\partial t} = (\pi e^2 / m^2 \sin^2 \alpha) (\partial / \partial \alpha) \{ (\sin \alpha B_k^2 / |v \cos \alpha|) (\partial f / \partial \alpha) \} \quad (3)$$

where $B_k^2 / 8\pi$ is the magnetic energy density of the whistler mode wave. Since $\partial f / \partial \alpha$ and $\partial f / \partial v_{||}$ are not independent, the resultant effect of the electron plasma and whistler mode waves on the electron distribution in the range where V_R and v_{res} overlap can be obtained by solving equations (1) and (3) simultaneously. Exact analytical solution of these equations is, however, very difficult and therefore, it would be worthwhile to look qualitatively into the behaviour of these equations.

For simplicity, only the initial stage of the growth of the whistler mode perturbation is considered, when its amplitude is too small to affect the background distribution significantly. For $\omega \ll \Omega$, $\gamma_c \propto A(V_R)$ and V_R is always negative. For a monotonically decreasing f with increasing $|v_{||}|$ and $v_{||}$, equation (2) gives:

$$A(V_R) \propto \left[\int_0^\infty v_{||}^2 \partial v_{||} \{ v_{||} / |v_{||}| \} | \partial f / \partial v_{||} | - | \partial f / \partial v_{||} | \} \right]_{v_{||} = V_R} \quad (4)$$

For an initially isotropic distribution $A(V_R) = \gamma_c = 0$ at $t = 0$. The quasilinear diffusion process caused by electron plasma waves leads to the formation of a ledge in f over the range of the resonant velocities. During this process $| \partial f / \partial v_{||} |$ diminishes at the centre but develops peaks on either side of the ledge (Fig. 1), which grow in height and drift in position towards the boundaries of the disturbed region (Fig. 2). As $\partial f / \partial v_{||}$ does not change during Landau resonance, the changes

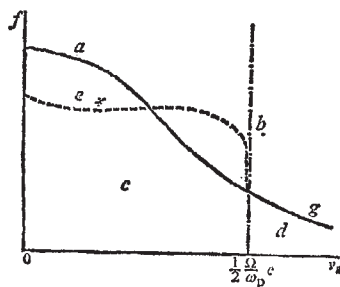


Fig. 3 a , f against $v_{||}$ at $t = 0$, b , largest $v_{||}$ for Landau resonance; c and d , the resulting disturbed and undisturbed regions, respectively; e , the ledge formed over c after quasilinear relaxation; g unchanged f over d . The steep gradient, $\partial f / \partial v_{||}$, that develops near $v_{||} = c\Omega / 2\omega_p$ would increase the pitch angle anisotropy and lead to whistler growth.

in $A(V_R)$ and γ_c (at corresponding frequencies) would follow those in $| \partial f / \partial v_{||} |$.

If ω is not much less than Ω , then γ_c will not become positive unless $A(V_R) > B$, which means that in the initial stage Landau damping would be accompanied with the cyclotron damping, and only after a certain time, when $A(V_R)$ exceeds B , would cyclotron growth occur.

This mechanism may play a significant role in the generation of natural and artificially stimulated very low frequency (v.l.f.) emissions. If a wide spectrum of oblique whistler turbulence³ exists in the magnetosphere for a period of the order of the Landau resonant quasilinear relaxation time, a ledge is likely to form over the entire disturbed region in the velocity space. The whistler dispersion $c^2 k^2 / \omega^2 = \omega_p^2 / \{ \omega (\Omega \cos \theta - \omega) \}$ relation shows that the phase velocity has a maximum, $c\Omega / 2\omega_p$, at $\omega = \Omega / 2$. Therefore, a particle with $v_{||}$ greater than this would not be in Landau resonance with the waves and would, consequently, lie outside the disturbed region (Fig. 3). A particle with $v_{||} = c\Omega / 2\omega_p$ would gyroresonate with a whistler mode wave with a frequency of $\omega = \Omega / 2$, propagating anti-parallel to B_0 .

The steep gradient in the curve of f against $v_{||}$ which is consequently formed at $v_{||} = c\Omega / 2\omega_p$ would, therefore, be unstable to whistler mode perturbations at $\omega = \Omega / 2$ propagating in the negative z direction as equation (4) suggests and wave amplification may be expected at this frequency. This amplification would, however, be delayed as $A(V_R)$ would take some time to exceed B in order to give a positive growth rate. The nature of these results would not be altered even if the initial distribution were assumed to have an anisotropy in pitch angle.

Very low frequency emissions occur more often at half the equatorial gyrofrequency than at other frequencies³; it is likely that the mechanism described here is one that favours emissions at that frequency.

NAND KISHORE VYAS
ANATH CHANDRA DAS

Physical Research Laboratory,
Ahmedabad 380009, India

Received August 6, 1974.

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Origin of the Naturaliste Plateau

LYING off the south-west coast of Australia (Fig. 1) some 2,500 m below sea level is the broad, rectangular Naturaliste Plateau, which forms a relatively flat ledge half way between sea level and the abyssal plain. How the plateau came to be in this position, and whether its origin is continental or oceanic, are two questions relevant to the evolution of the south-east Indian Ocean.

Until recently the only data bearing on the origin of the plateau were seismic profiles recorded by RV Vema¹ and by RV Robert D. Conrad, which also obtained 2 m of Upper Cretaceous sedimentary rock² by gravity coring (RC8–56). Deeper core information was obtained in late 1972 by the Glomar Challenger at Deep Sea Drilling Project sites 258 and 264 (refs 3 and 4, and Fig. 2). Preliminary study of DSDP data indicates that the plateau has been at its present depth since Early Cretaceous (Middle Albian) time, accumulating pelagic sediments, and 'has probably been uplifted since that time rather than having subsided'³.

DSDP 258 penetrated 525 m of marine sediment up to 105 Myr old, containing detrital units in the Early Cretaceous which may be volcanically derived and which support an oceanic origin. But a Miocene/Santonian unconformity

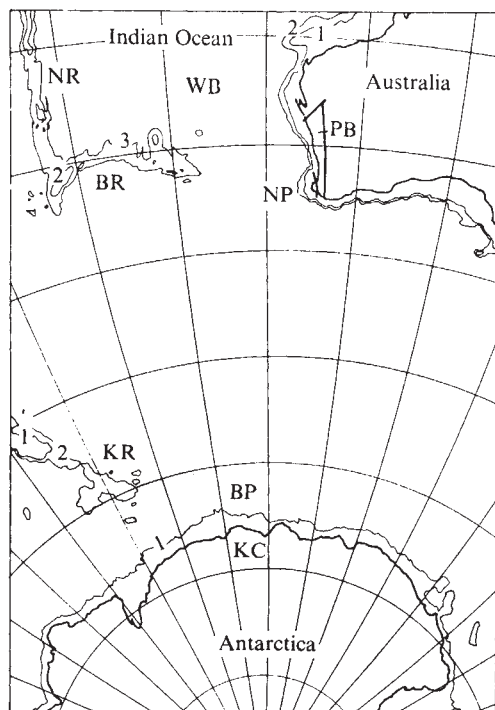


Fig. 1 Location of the Naturaliste Plateau (NP) in the south-east Indian Ocean. BR, Broken Ridge; NR, Ninety-east Ridge; WB, Wharton Basin; KC, Knox Coast, BP, Bruce Plateau; KR, Kerguelen Ridge; PB, Perth Basin. Bathymetric contours in km after McKenzie and Sclater¹¹.

which represents a break of 70 Myr was encountered only 100 m below the seafloor and may be related to the final separation of Australia and Antarctica in Middle Eocene time. In the Robert D. Conrad core (RC8-56) an unconformity separates Pleistocene and Turonian beds.

DSDP 264 near the southern edge of the plateau penetrated 215 m of carbonate sediment and bottomed in an undated volcanoclastic conglomerate containing both acid and basic volcanic rocks. The Tertiary/Cretaceous unconformity at the other core sites is here represented by three unconformities spanning late Miocene/late Eocene, early Eocene/mid-Palaeocene, and mid-Palaeocene/Cenomanian times. The erosion causing these shallow unconformities seems to have been less severe at the margins of the Plateau, as evidenced by the presence of Eocene and Palaeocene sections at site 264.

The first systematic survey of the area was achieved when the Australian Bureau of Mineral Resources (BMR) completed a series of 13 profiles over the plateau in December 1972. This was part of BMR's survey of the Australian continental margin using continuous gravity, magnetic, seismic, and bathymetric recording at a line spacing of 30 nautical miles. A preliminary study of these data together with previous information suggests a continental origin for the plateau.

The western and eastern halves of the Naturaliste Plateau differ significantly in bathymetric, seismic, and magnetic characteristics. The western half is the shallower and is characterised by shallow seismic basement and intense magnetic anomalies. The basin-like eastern area has thick sediments and less intense magnetic anomalies. Along the southern and parts of the western margin, but not along the northern margin, scarp-like bathymetric features suggest faulting in the basement. Elsewhere the seismic results show that the plateau sediments are not faulted or folded. The thickest sedimentary sequence is in the north-west

corner and in the low area to the east, where at least 2 km of sediment is present.

The Tertiary/Cretaceous unconformity is too close to the sediment-water interface at drilling sites 258 and 264 for detection on BMR seismic records. But three deeper unconformities were revealed. All four (A, B, C, and D) are shown on the cross section along XX' shown in Fig. 3. The Tertiary/Cretaceous unconformity (A), detected in all three plateau cores, also occurs at Broken Ridge and Wharton Basin Sites³, and is of regional extent. Unconformity B extends over the whole plateau and truncates unconformity C, restricting it to the eastern part. The sediment/acoustic basement unconformity (D) is most readily identifiable in the areas of thin sediments in the west.

The well stratified sequence above unconformity B constitutes about half the total volume of sediments evident on the plateau and shows several local unconformities. Its thickness ranges from 200 to 1,100 m, assuming a sediment seismic velocity of 2.0 km^{-1} and DSDP data indicate that it is largely of Cretaceous age with only about 100 m of

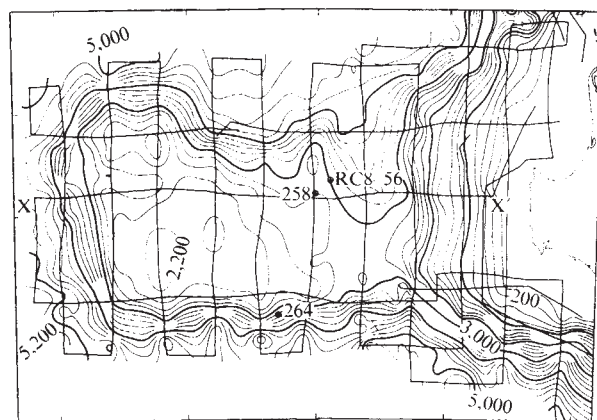


Fig. 2 Bathymetry of Naturaliste Plateau, BMR traverses and core locations. RC8-56, core by RV Robert D. Conrad; 258, DSDP core site 258; 264, DSDP core site 264. Bathymetric contour interval 200 m.

Miocene and younger beds. Most sedimentation on the plateau, therefore, preceded the separation of Antarctica and Australia in Eocene time.

DSDP 258 was near the pinch out between unconformities B and C, and bottomed just short of C in a thick sequence of Albian clays and glauconitic sands. Thus, the beds between B and C, which reach a thickness of 700 m in the deep eastern area, are evidently these Albian clays. On the seismic sections they are largely transparent, but stratification becomes increasingly evident northwards.

Unconformity C seems likely to be Neocomian because it is close to the Albian clays at the bottom of DSDP 258 and because prominent prior tectonic activity known in the area took place in the Perth basin within Neocomian time. Jones and Pearson⁵ believe this to be the time when India became detached from the west coast of Australia during the breakup of Gondwanaland. But recent DSDP data from site 259 in the Perth Abyssal Plain indicated that this fracturing continued throughout the Cretaceous⁶. The basalt at the base of DSDP 259 was dated as Neocomian.

Below unconformities B in the west and C in the east are well stratified sediments ranging in thickness from 0 to 1,000 m. In the west they are draped over the basement, in the east they extend to the limit of seismic penetration. They are only gently folded and faulted, and were deposited before the Cretaceous activity.

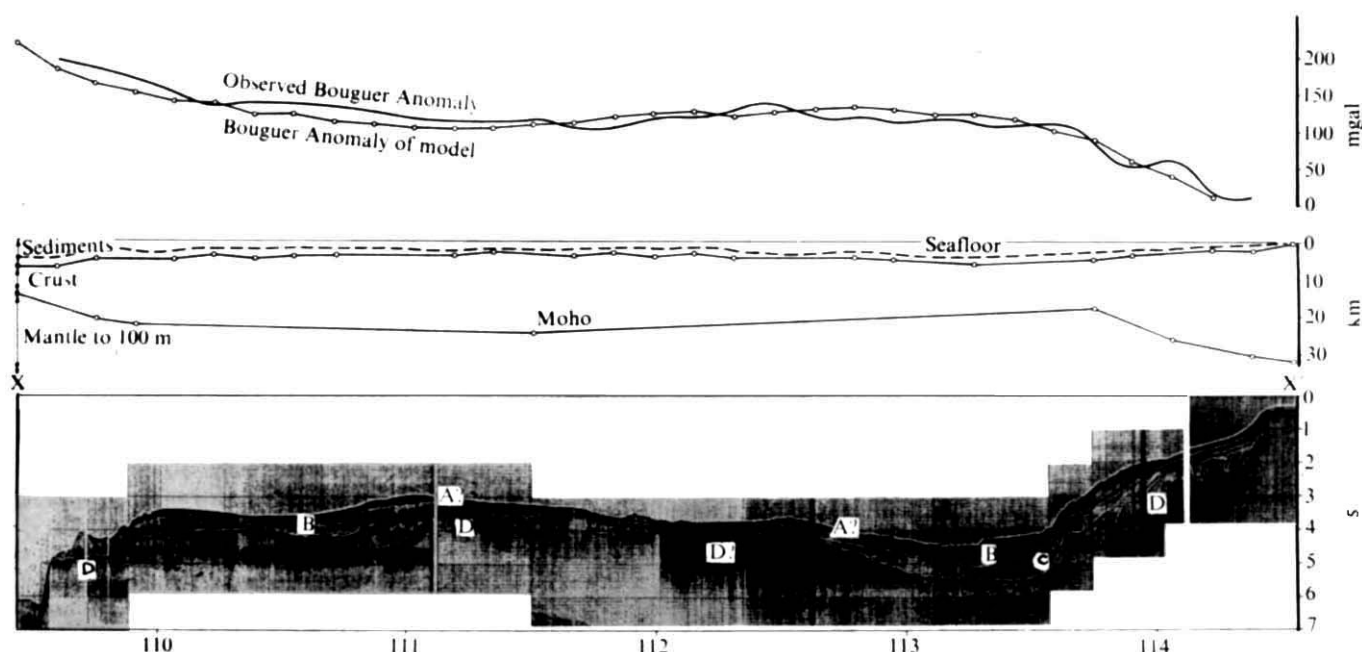


Fig. 3 Bouguer anomaly crustal model of the Naturaliste Plateau and seismic interpretation for section XX'. Densities used in gravity model: mantle 3.45 g cm^{-3} ; crust, 2.85; sediments, 2.20. Standard crust for south-west Australia taken as 31 km thick after Mathur¹². A, B, C, D, are unconformities. ↓ DSOP site 258.

Acoustic basement is more visible on the seismic records from the western parts of the plateau, where sediments are generally thinner than in the east. Basement topography resembles a block-faulted and eroded plateau. Occasional reflection events below the basement unconformity suggest that the sequence is sedimentary, and this may be supported by the lack of strong reflectivity contrast with overlying sediments in some areas. Elsewhere there are features in the seismic sections showing intrusive form.

The magnetic anomaly pattern is more disturbed in the west, indicating a shallower magnetic basement than in the east. The high frequency and amplitude of magnetic features in the west suggest that the basement in this region is a complex of basic igneous and metamorphic rocks. This, together with the seismic evidence, points to a basement of metamorphosed sedimentary rock with basic igneous intrusions.

The Bouguer anomaly model showing the best fit with observed gravity is illustrated in Fig. 3: crustal thickness is about 22 km, intermediate between continental and oceanic thicknesses, and is a further indication that the plateau was not originally an oceanic feature. A similar thickness was found⁷ under Broken Ridge from seismic refraction surveying in 1962 and it was suggested then that this feature is continental and may have been contiguous in the past with the Naturaliste Plateau and the Western Australian Shield.

BMR and DSDP data indicate that most of the plateau sedimentation preceded the separation of Australia and Antarctica and a large part was almost certainly pre-Cretaceous. The sediments were deposited on a basement of metamorphic rocks, and have remained tectonically undisturbed except along the southern and western margins. It is concluded that the plateau is of continental origin and existed as part of the continental mass of Gondwanaland in essentially the same position relative to Australia that it occupies today. Location of marginal scarp features suggests that there were landmasses along its southern and western margins, but not along its northern margin.

The reconstruction of Gondwanaland by Veevers *et al.*⁸ places India against the south-west coast of Australia and the Naturaliste Plateau some 500 km north of its present position. The reconstruction was based on evidence that the Perth Basin was within the interior of Gondwanaland up to the earliest Cretaceous. The BMR and DSDP data cast doubts on this reconstruction. The western basement block of the plateau could not have moved great distances horizontally relative to Australia since the deposition of the sediments without causing sedimentary deformations in the east that would be unmistakable in seismic records.

In the fit of Australia and Antarctica proposed by Sprall and Dietz⁹ the Naturaliste Plateau lies against a similar Antarctic marginal bathymetric feature known as the Bruce Plateau, offshore from Knox Coast. Rocks outcropping on Knox Coast are shown on the Geological Map of Antarctica¹⁰ to be Precambrian gneisses and schists of granulite facies. As suggested by the geophysical evidence such rocks are more likely than oceanic igneous rocks to form the basement of the Naturaliste Plateau. There is reason to believe, therefore, that the plateau once formed part of the Gondwanaland continental shelf (which explains the intermediate crustal thickness) abutting the Bruce Plateau. It broke away from Antarctica in the initial rifting which formed the southern Australian continental scarps and thereafter it remained attached to the Australian crustal plate.

PETER PETKOVIC

Australian Bureau of Mineral Resources,
PO Box 378, Canberra, Australia

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The contribution of palaeosoil to Egyptian lithostratigraphy

THOROUGH examination of the contact between the basement complex (mainly Precambrian) and the overlying Nubia Sandstone (Upper Cretaceous) in seven localities in the Eastern Desert of Egypt (Fig. 1) has revealed the existence of a well defined lateritic palaeosoil capping the former rocks. Previously, mention has only been made¹ of a local kaolinised surface of the basement rocks and the lateritic palaeosoil has been neglected in the lithostratigraphy^{2–4}. The nature, thickness (4–10 m remaining after erosion) and the lateral extent of the soil over a wide variety of rock types necessitates, however, the introduction of a separate unit, which we suggest is called the 'I'byan Soil'. This palaeosoil may cover a large area of Egypt and we suggest that the same nomenclature is used at all localities where the soil has lateritic affinities.

At the type locality, about 1 km north of the 97-km post east of Idfu (longitude 33° 44'E and latitude 25° 2' 30"N), the I'byan Soil is 10 m thick and caps the Barramiya Serpentinities (Fig. 2). In other localities it caps either old volcanics (Fig. 1, locality 2), arenites of the

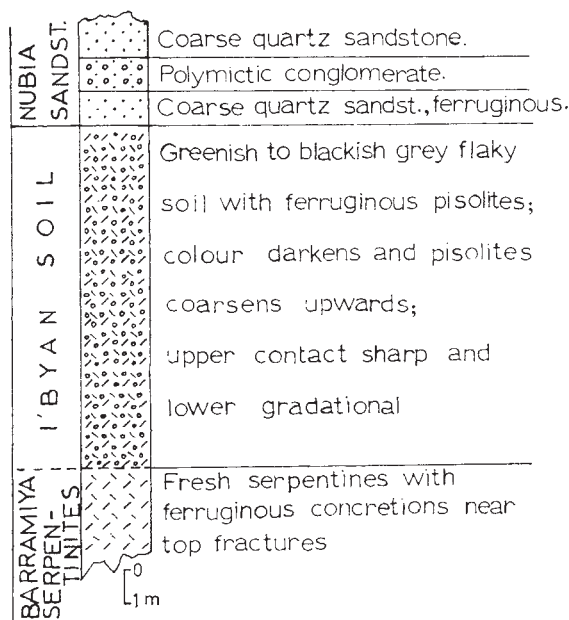


Fig. 2 The succession at the type locality.

Hammamat Group (Fig. 1, locality 3), granodiorite (Fig. 1, locality 4) or metasediments and metavolcanics (Fig. 1, localities 5–7). The exact nature of the weathering of the basement rocks before the sandstone was deposited is now being studied through detailed geochemical and mineralogical analyses.

E. RAMZY PHILOBBOS*
 KAMAL EL-DIN K. HASSAN

Department of Geology,
 Assiut University,
 Assiut, Egypt

Received October 11, 1974.

* Present address: Royal School of Mines, Imperial College of Science and Technology, London SW7 2BP, UK.

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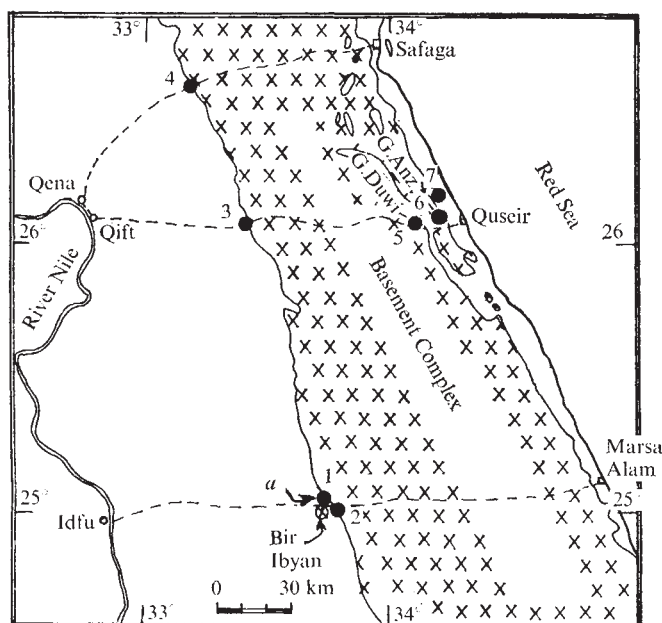


Fig. 1 Locality map showing the visited localities of the 'I'byan Soil'. Roads are shown as dashed lines. a, Type locality.

Decreased global rainfall during the past Ice Age

WE have made a synthesis of atmospheric conditions for 20,000 yr BP based on fairly standard palaeoclimatological data and procedures. From mean zonal conditions at 20° latitude intervals from 80°N to 80°S we have computed radiative heating rates and the energy balance of atmospheric columns. We find that net cooling of the atmosphere by radiative processes was smaller at 20,000 yr BP than it is now. The total heating of the air, which sums to zero in the annual average except during periods of climatic change, is the sum of heating by latent heat liberation, heating by conduction and convection from the boundary layer, and radiative cooling (see for example Fig. 7 in ref. 1). Because the change in radiative cooling is comparable to boundary layer heating itself, we postulate that the decrease in cooling is accompanied primarily by a decrease in heating from rainfall and therefore that global rainfall was smaller at 20,000 yr BP than it is now. Examination of the magnitudes of the three components suggests that the decrease in rainfall was about 10%.

Details of the palaeoclimatic reconstructions will be published elsewhere but for completeness we state here the chief assumptions made and give the radiative cooling rates found.

Surface air temperature maps for January and July were synthesised from observation of oxygen isotopes in ice cores, observations of tree lines, snow lines, pollen distributions and foraminifera distributions. Variation of temperature in the vertical was assumed to have the same form as today. Specific humidity was taken as a function of temperature only, the relationship in the troposphere being based on present-day observations. Stratospheric moisture and ozone concentrations were taken as identical to present-day values. Carbon dioxide was assumed to have a uniform distribution of 320 parts per million by volume. Cloud was assumed to be absent everywhere. A set of zonally averaged albedo values was drawn up from a consideration of the nature of the surface at each point on a $10^\circ \times 10^\circ$ grid. Radiative heating rates, containing contributions from both the solar and terrestrial portions of the spectrum, were computed following the technique devised by Rodgers² and modified by Dopplick³. Meridional cross sections of these rates were drawn up and integrations performed to obtain the total cooling of the air by radiative processes. These integral values, for the present atmosphere with cloud and without cloud and for the 20,000 yr BP synthesis without cloud, but with and without the albedo change, are shown in Table 1.

Table 1 Integral over the entire atmosphere of the net radiative cooling rate

	Present day		20,000 yr BP	
	With cloud	Without cloud	No albedo change	New albedo
January				
Globe	10.4	11.2	10.6	10.4
Northern Hemisphere	5.9	6.1	5.6	5.6
Southern Hemisphere	4.5	5.1	5.0	4.8
July				
Globe	10.5	13.1	11.8	11.7
Northern Hemisphere	4.6	6.7	5.5	5.3
Southern Hemisphere	5.9	6.4	6.3	6.4

Units: 10^{20} calorie d^{-1} .

The present-day values show greater cooling without cloud than with cloud. Inspection of the cross sections shows that when the cloud is removed some regions at middle latitudes alter from slight radiative heating to radiative cooling. It therefore seems reasonable to interpret this difference as due to the blanketing effect of the cloud. When we compare the results without cloud for 20,000 yr BP and the present, it is evident that there is a smaller net cooling at 20,000 yr BP, substantially all due to changes in the Northern Hemisphere. Most of this change is due to temperature and specific humidity changes, rather than to albedo changes. The latter influences the solar input radiation to the ground-atmosphere system but has no effect on the terrestrial radiation lost to space. Treating the air alone, there is less cooling in the 20,000 yr BP synthesis because temperature and moisture content were both lower.

The air itself is able to maintain an annual equilibrium temperature because the net radiative cooling is counteracted by latent heat liberation and boundary layer heating (see Fig. 7 of ref. 1). These values are respectively about 9.0 and 1.5×10^{20} calorie d^{-1} for the present day atmosphere. A decrease in radiative cooling of about 1.0×10^{20} calorie d^{-1} , for the 20,000 yr BP synthesis, must have been accompanied by a decrease in net heating by the same amount. We postulate that a large fraction of this decrease occurred in the latent heat liberation term and that the rainfall was therefore smaller, by roughly 10%. The values in Table 1 suggest that most of this change occurred in the Northern Hemisphere.

There have been many discussions in the past when it was argued that Ice Ages were 'pluvials,' which had the connotation of additional rainfall. The above comments suggest that rainfall was less then than now. Recently some palynological records from the tropics have also been interpreted to imply that 20,000 yr BP was actually a drier time than the present⁴.

Kraus⁵ has also argued for a decreased rainfall based upon a presumed lower evaporation at 20,000 yr BP than at present; our argument reaches the same conclusion from an independent line of reasoning.

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REGINALD E. NEWELL
GERALD F. HERMAN
SHARON GOULD-STEWART
MINORU TANAKA

Department of Meteorology, 54-1520,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received October 31, 1974.

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Glacial climates in the eastern tropical South Pacific

RECENT attempts to explain the glacial aridity at upper elevations in the Galapagos Islands have failed to take into account either concurrent events in neighbouring areas¹⁻⁶ or modern causes of climate. Yet, any reconstruction of the glacial climatology of the South Pacific must be consistent with all these data. Recognised glacial features of the region that must be taken into consideration and the empirical evidence which supports them include: increased upwelling along the equatorial divergence (enhanced plankton production⁷, simultaneous build up of guano⁸), upper elevation (650 m) aridity relative to the present in the Galapagos Islands (dated palaeomicrofloras³); increased precipitation above 2,000 m along the western slopes of the Peruvian Andes (deposits of oxides^{9,10}, traces of humid cycle stream cutting and scouring of alluvium⁹⁻¹¹, remains of sheet flooding at the base of the mountains^{9,10} a disproportionate lowering of the west facing glacial snowline¹², relics of formerly more continuous plant and animal distributions^{13,14}); continuous aridity throughout the Pleistocene along the Peruvian coast (unbroken sequence of arid cycle processes⁹⁻¹¹); and exposure of large areas of the western Indo-Australian region following the 100-120 m sea level drop¹⁶ (fossil terraces^{16,17}). It can be deduced from the position of this exposed land that the modern pattern of vernal mixing of water from the eastern and western Pacific could not have taken place¹⁷ and that the eastern Pacific anticyclone did not, therefore, weaken in the glacial summer¹⁷. Thus, the pressure gradients between the two sides of the ocean would have remained fixed throughout the year and the eastern anticyclone and the south-east trade winds would have retained high intensity in both summer and winter.

At present, the low elevations of the Galapagos receive no moisture for most of the year and the upper elevations (1,000-1,700 m) are at best, covered by fog^{1,18,19}. The fog zone is the result of a temperature inversion brought about

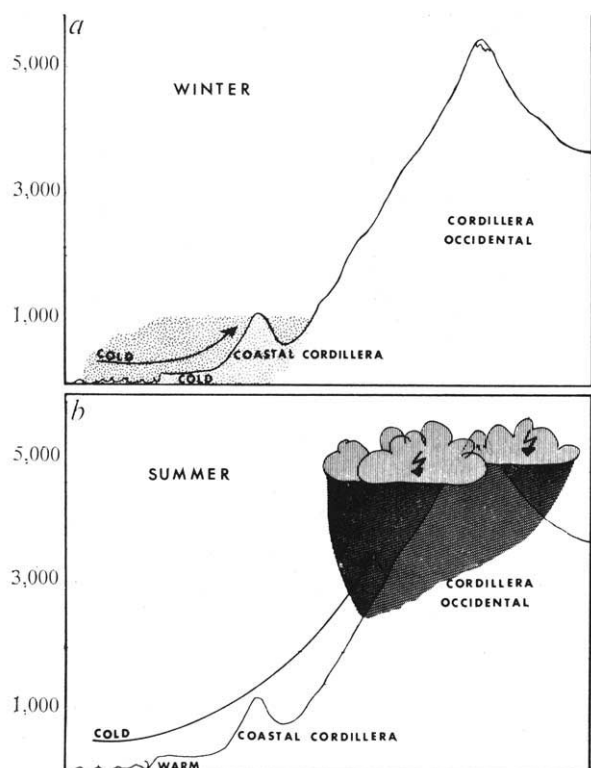


Fig. 1 Schematic representation of modern day climate along the coast of Peru at approximately 12°S latitude (adapted from Bowman²²). *a*, Winter conditions with garua (fog) along the coast, and Andean summits clear; *b*, summer conditions with thundershowers and rain at high elevations and clear weather along the coast.

by the cooling, compression and drag of the lower part of the air mass crossing the cold equatorial upwelling on its way to the Galapagos^{18,19}. Rains occur in the spring when the eastern Pacific pressure system weakens and permits the invasion of warm air masses from the north. This southern penetration of warm northern air constitutes the normal summer movement of the Intertropical Convergence Zone (ITCZ). An abnormally pronounced penetration and its accompanying storms is commonly called 'El Niño'^{20,21}.

In western Peru, modern rainfall patterns are also seasonal but are caused by semiannual changes in the temperatures of the land (t_l) relative to that of the bordering ocean (t_o). In the southern winter, onshore winds are very cold, having blown across the Peru Current and the coastal upwelling²⁰⁻²². These winds hit an equally cold or colder coast ($-3^\circ\text{C} < t_l - t_o < 1.5^\circ\text{C}$) lose their velocity and essentially stagnate in the form of the famous garua (fog) of the coastal hills²² (Fig. 1*a*). Throughout this period, the high slopes of the Cordillera are sunny and dry. During the southern summer, the onshore winds are only slightly warmer than in winter (between 1° and 4°C , see refs 20, 21, 22) because they still cross the Peru Current and the coastal upwelling. The coast, however, heats up considerably (2° – 6°C) as the Sun moves south, resulting in a large land-ocean temperature difference ($1^\circ\text{C} < t_l - t_o < 6^\circ\text{C}$). The net effect of the cold onshore breezes hitting the warm coast is that the air is heated, absorbs energy and increases in velocity as it moves toward the nearby Andes. The strength with which the air hits the mountains triggers an unstable rapid rise. Condensation and thundershowers occur when the air reaches about 2,000 m (Fig. 1*b*). In contrast to the fogs of winter, summer is clear along the coast.

The simplest model of glacial climatology should explain all of the ice-age features in the tropical South Pacific without requiring any *ad hoc* postulations of phenomena beyond those currently operative. Yet previous hypotheses

disregarded the implications for neighbouring areas or theorised major changes in the climatic pattern. Thus, the suggestion that the increase in moisture along the upper Andean slopes was caused by a weakening of the Peru Current^{9,10} or the coastal upwelling⁸ is unsubstantiated and overlooks the fact that decreases in the strengths of these currents would have allowed an invasion of warm water from the north and caused increased precipitation in the Galapagos and along coastal Peru. A similar hypothesis³, that the glacial ITCZ remained south of the equator throughout the year, is inconsistent with arid conditions in the Galapagos and along the coast of Peru. The converse theory, that the ITCZ remained north of the equator during the year¹ does not seem likely on theoretical grounds³ and would have caused dry 'winter' conditions to prevail for almost 12 months along the high Andes. A recent analysis of correlations between surface sea temperature and the lack of rainfall in the Galapagos Islands led to the postulation that increased upwelling at the zone of equatorial divergence caused the aridity of the islands², but the study did not explain the causes for increased upwelling and similar reasoning was not extended to areas outside the zone of the divergence.

The model proposed here incorporates this most recent observation, accounts for all of the documented glacial events in the tropical South Pacific, allows for local climatic anomalies near upwellings and is based solely on substantiated changes in atmospheric conditions and modern patterns of climate.

Wind intensity and high atmospheric pressure were maintained throughout the year because of the prevention of the summer relaxation of the eastern Pacific anticyclone¹⁷ and the northward movement of the westerlies⁴⁻⁸ which resulted in compression of the south-east trade wind system. This year long maintenance of high atmospheric pressure and intense circulation led to correspondingly intense ocean currents and vigorous upwellings at the zone of equatorial divergence and along the Peruvian coast. Thus, waters as cold as, or colder than, those of the present winter were being brought to the surface or from higher latitudes near the Antarctic Ice Cap continuously throughout the year. Estimates from changes in carbonate composition and in the distributions of foraminifera^{23,24} indicate a cooling of ocean waters (5° – 6°C) off the coast of Peru. Estimates of low latitude, low elevation, land surface temperature depression are usually less than 3°C (ref. 16). Along the Peruvian coast, unequal land-ocean cooling would have meant that temperature differences in a glacial winter would have approximated the present summer difference ($1^\circ\text{C} < t_l - t_o$). The modern summer pattern of arid lowlands and thundershowers at upper elevations, would have continued during most of the year. A corresponding increase in aridity at upper elevations in the Galapagos produced by lowered sea surface temperatures along the equatorial divergence was discussed by Houvenaghel².

It must be emphasised, however, that in the tropics, most climatic changes probably occurred near the beginning or the end of traditional temperate latitude glacial cycles, when ocean and land surface temperature differences were at a maximum and caused the most important effects of the Pleistocene in low latitudes—repetitive cycles of aridity and humidity¹³.

BERYL B. SIMPSON

US Museum of Natural History,
Smithsonian Institution,
Washington, DC 20560

Received November 4, 1974.

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Interaction of sodium dodecyl sulphate and polyethylene oxide

AN increase in the concentration of K_2CO_3 in Polyox WSR 205 causes a decrease in the drag reduction¹; this has been successfully correlated with lowered values of the intrinsic viscosity¹. Nonionic, water-soluble polypeptides can interact with sodium dodecyl sulphate (SDS) to form complexes², and above a certain concentration the reduced viscosity of some of these polymers is increased. The immediate implication is that if such interactions also take place with polyethylene oxides of high molecular weight then changes in the observed drag reduction may occur.

Some sort of soap-polymer interaction does occur (Fig. 1); at all polymer concentrations the drag reduction is significantly improved in the surfactant solution. The turbulent flow line of SDS alone was not measurably different from that of pure water. This seems to be the first evidence that the drag reduction of a polymer in a good solvent can be slightly improved by the addition of a third component to the system. For example, at intermediate concentrations of 7-10 parts per million (p.p.m.) of Polyox WSR 205 there is a 35% increase in the observed drag reduction (Fig. 1). The observed increase in drag reduction is similar to that which occurs at the lower Reynolds number of 6,000 (Fig. 2). The increase in percent drag reduction over a similar concentration range is again of the order of 35%. The decreased effectiveness of the polymer-soap complex as the Reynolds number, Re , is increased is summarised in Table 1 where the intrinsic concentration, $[c]$, provides a measure of the efficiency of the drag reduction. It is obvious that the large increase in intrinsic concentration for the polymer-soap complex as compared with the polymer alone suggests that the complex

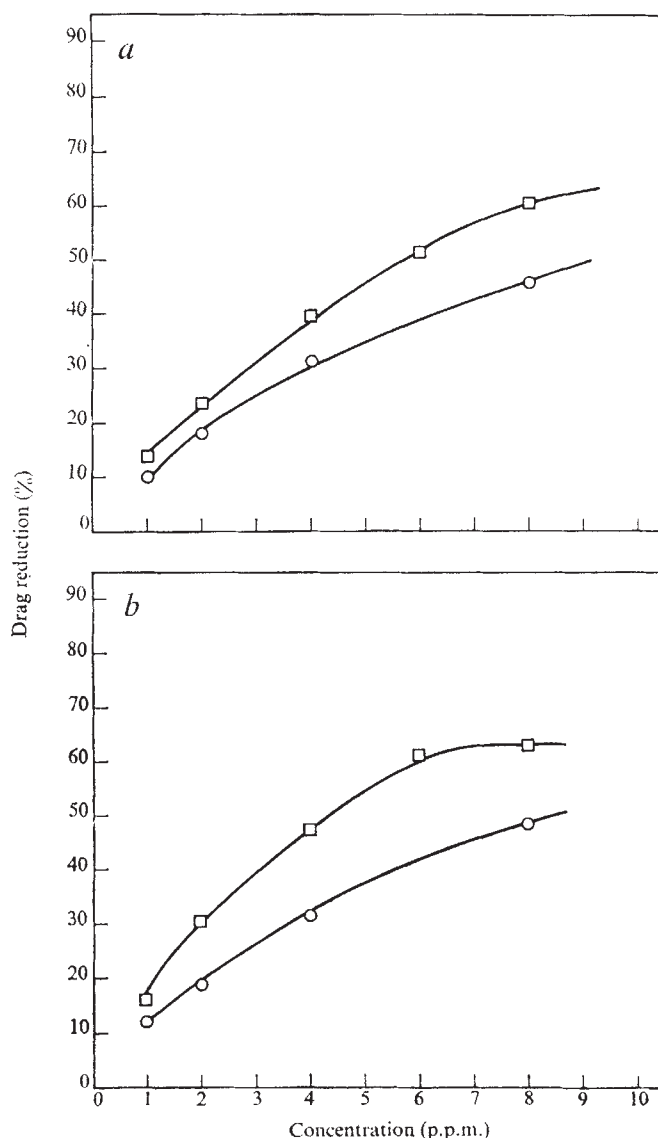


Fig. 1 Drag reduction against concentration for Polyox WSR 205 in water (O) and 0.2% SDS solutions (□). a, At $Re = 9,000$; b, at $Re = 6,000$.

is breaking up at higher shear rates or stresses. This may not be too surprising in itself as aqueous drag reducing soap systems involving additional simple substances such as electrolytes³ and certain amines⁴ have exhibited stress-controlled drag reduction.

A ready explanation for this polymer-surfactant behaviour has been suggested². Apparently, in the present case hydrophobic bonding takes place between the polymer and the hydrocarbon tail of the surfactant. As sufficient surfactant becomes bonded to the polymer, electrostatic repulsion between bound surfactant ions on the polymer backbone causes the polymer coil to expand. Essentially, then, in the present case the equivalent of a polyelectrolyte is created through polymer-surfactant interaction. The dependence of such interactions on the nature of the surfactant and the polymer will be the subject of future work.

R. L. PATTERSON
R. C. LITTLE

Naval Research Laboratory,
Washington, DC 20375

Received September 18, 1974.

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² Murai, N., Makino, S., and Shintaro, S., *J. Colloid Interface Sci.*, **41**, 399 (1972).

Table 1 Effect of Reynolds number on complex

Solvent	$[c]$, $Re = 6,000$	$[c]$, $Re = 9,000$
H ₂ O	8.5	8.9
0.20% SDS	5.6	7.6

³ Savins, J. G., in *Viscous Drag Reduction* (edit. by Wells, C. S.), 183 (Plenum Press, New York, 1969).

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Bond energy of the IO radical from molecular beam reactive scattering measurements

THE bond energy $D_0(\text{IO})$ of the IO radical is not reliably known. An early estimate, $D_0(\text{IO}) = 44 \text{ kcalorie mol}^{-1}$, was derived by Gaydon¹ from the IO emission spectrum of the CH_3I flame. Since this value was obtained by Birge-Sponer extrapolation of only five vibrational levels of the upper $\text{IO}(^2\Pi)$ state, it is not expected to be very accurate. The same spectrum was studied by Ramsay² in absorption by flash photolysis of O_2/I_2 mixtures. Birge-Sponer extrapolation of six bands gave $D_0(\text{IO}) = 42 \pm 5 \text{ kcalorie mol}^{-1}$. But more accurate extrapolation² for ClO and BrO using twenty bands showed that extrapolation from a small number of low lying levels leads to unreliable results. A rigorous upper bound $D_0(\text{IO}) < 62.8 \text{ kcalorie mol}^{-1}$ was determined from predissociation of the upper $\text{IO}(^2\Pi)$ state.

A value $D_0(\text{IO}) = 57 \pm 6 \text{ kcalorie mol}^{-1}$ was estimated by Sugden³ by photometry of the IO emission spectrum from a $\text{O}_2/\text{H}_2/\text{I}_2$ (1%) flame equilibrium. Two estimates^{4,5} have been made by extrapolation of the RKR potential curve of the upper $\text{IO}(^2\Pi)$ state with model potentials; one⁴ supporting the higher flame equilibrium value and the other⁵ supporting the lower Birge-Sponer value.

Recently, preliminary reports⁶⁻⁸ of O atom reactive scattering by ICl have shown an important reaction path to be



In our experiments, a crossed molecular beam apparatus⁹ with an electron bombardment mass spectrometer detector was used. The O atom beam was produced from a low pressure (~ 1 torr) microwave discharge through O_2 . The discharge region was separated from the beam source slit by ~ 60 cm of Pyrex tube. This configuration results in a low temperature ~ 350 K atom beam and suppresses $\text{O}_2(^1\Sigma_g^+)$ metastable molecules. The ICl cross beam issues from a supersonic nozzle source and has a narrow velocity distribution ($\sim 20\%$ full

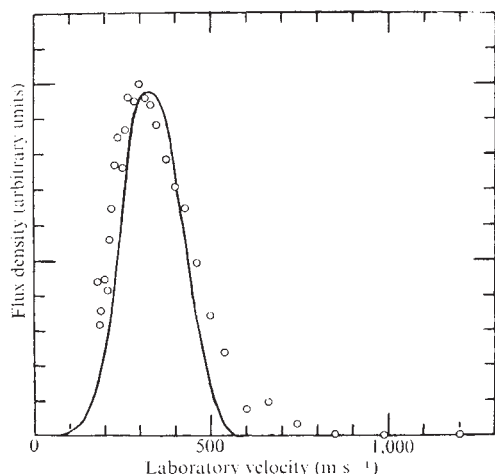


Fig. 1 Laboratory velocity distribution (flux density) of IO reaction product along the direction of the nominal centroid vector. $\text{O} + \text{ICl} \rightarrow \text{OI} + \text{Cl}$. $\Theta = 79^\circ$.

width at half maximum). The most probable reactant translational energy was $E = 0.8 \text{ kcalorie mol}^{-1}$. The distributions of IO product velocities have been measured by a computer time-of-flight system over a range of laboratory scattering angles. Analysis of the full experimental data shows that the reaction proceeds by a long-lived complex mechanism.

The distribution of IO product translation energy in centre of mass coordinates is most accurately determined by observation of the IO velocity distribution along the direction of the centroid vector in laboratory coordinates. The IO flux density distribution measured at laboratory scattering angle $\Theta = 79^\circ$ (nominal centroid vector at $\Theta = 78^\circ$) is shown by open circles in Fig. 1. The distribution for the long-lived collision complex, predicted by a model¹⁰ based on RRKM theory of unimolecular decomposition, is also shown by a solid curve in Fig. 1. Agreement with the experimental data is obtained for a reaction exoergicity $\Delta D_0 = 3.4 \text{ kcalorie mol}^{-1}$. Using¹¹ $D_0(\text{ICl}) = 49.6 \text{ kcalorie mol}^{-1}$, we obtain $D_0(\text{IO}) = 53 \pm 3 \text{ kcalorie mol}^{-1}$.

This value of $D_0(\text{IO})$ also gives good agreement with our time of flight measurement of the $\text{O} + \text{I}_2 \rightarrow \text{IO} + \text{I}$ reaction. But the exoergicity, $\Delta D_0 = 17.4 \text{ kcalorie mol}^{-1}$, is greater for this reaction. Thus it does not provide as sensitive a test of $D_0(\text{IO})$ as the almost thermoneutral $\text{O} + \text{ICl}$ reaction.

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D. ST. A. G. RADLEIN
J. C. WHITEHEAD
R. GRICE

Theoretical Chemistry Department,
Cambridge University,
Cambridge CB2 1EW, UK

Received October 21, 1974.

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Unique form of filamentous carbon

CONSIDERABLE interest has been shown in the formation of carbon filaments from the catalytic decomposition of gases over metal surfaces at about 700°C (see refs 1, 2). Detailed studies have been chiefly confined to the behaviour of pure metals, but technically and industrially the effect of alloying elements is also important. We have studied how additives to an iron catalyst modify the filament growth process during decomposition of acetylene. With acetylene decomposed on a Pt/Fe alloy a new mode of filament growth was identified in which the complete detachment of a catalyst particle from the surface of the metal was not a necessary prerequisite for growth³. Here we describe the effect of tin on the growth of

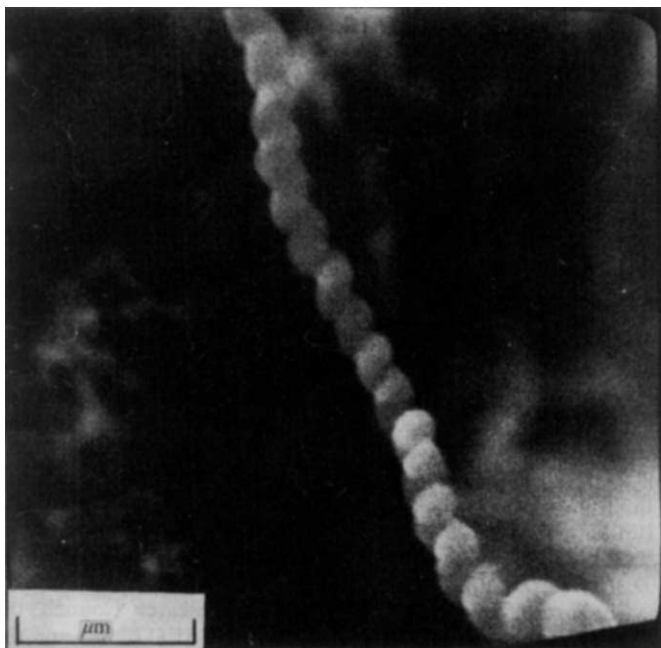


Fig. 1 Scanning micrograph of a spiral filament produced from Sn/Fe catalysed decomposition of 5 torr acetylene at 800°C.

filaments from the iron/acetylene system. The experimental technique used in this work, controlled atmosphere electron microscopy, has been described elsewhere⁴.

Significant deposit growth was only observed at temperatures above 800°C with tin coated iron foils in 5 torr acetylene and

was preceded by an induction period of about 10 min at this temperature. On pure iron, deposits grew at temperatures as low as 650°C under otherwise similar conditions. Pure tin did not produce a deposit. In contrast to the growth on pure iron, which became autolytically poisoned after a few minutes, growth still continued on Sn/Fe after more than 2 h and poisoning was never observed. The deposit was filamentous, a common form of growth from acetylene^{1,2}, but the filaments were nearly all spirals with a constant pitch (Fig 1). Although spiral filaments have been observed in other systems^{1,5,6} they are rare and the production almost exclusively as spirals is unusual.

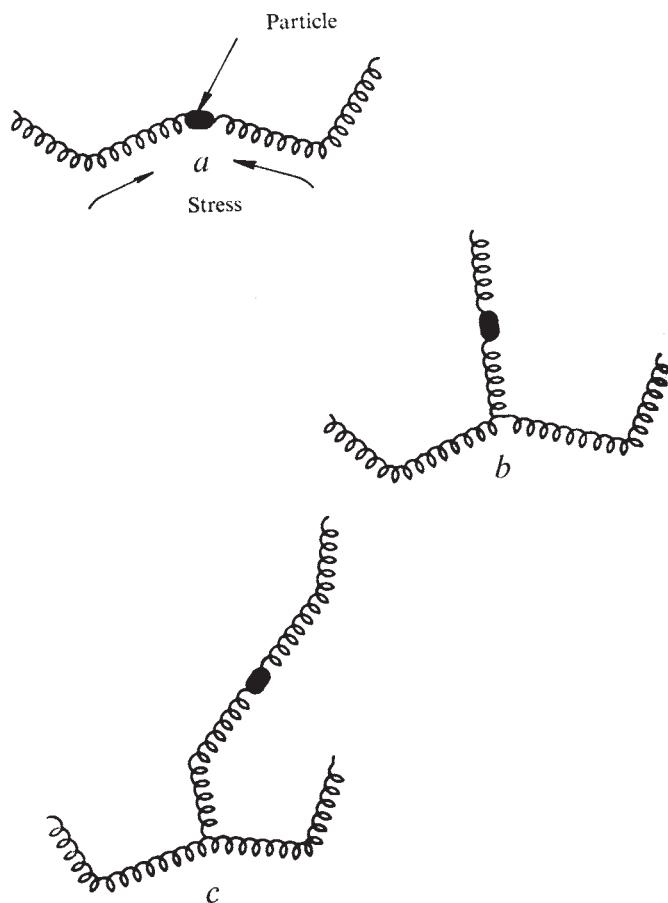


Fig. 3 Mode of formation of branched filaments.

The average pitch θ , can be obtained from a plot of the spiral length, l , against the diameter of the spiral, d ;

$$l = \pi d \tan \theta$$

and is found to be $30^\circ 41'$ (Fig. 2).

Two filaments grew from each particle in opposite directions and it was not possible to discern the shape of the particle. The two filaments grew at identical rates and any kinks or imperfections in one were reproduced in the other so that the two filaments were exact enantiomorphs except for the handedness of the spirals which, viewed from the particle, were opposite handed in every case. Branching of filaments was common and seemed to be the principal means of relieving the stress created by the fact that filaments were often forced against the mass of deposit in two directions. When this stress became too great the particle was squeezed out from between these two

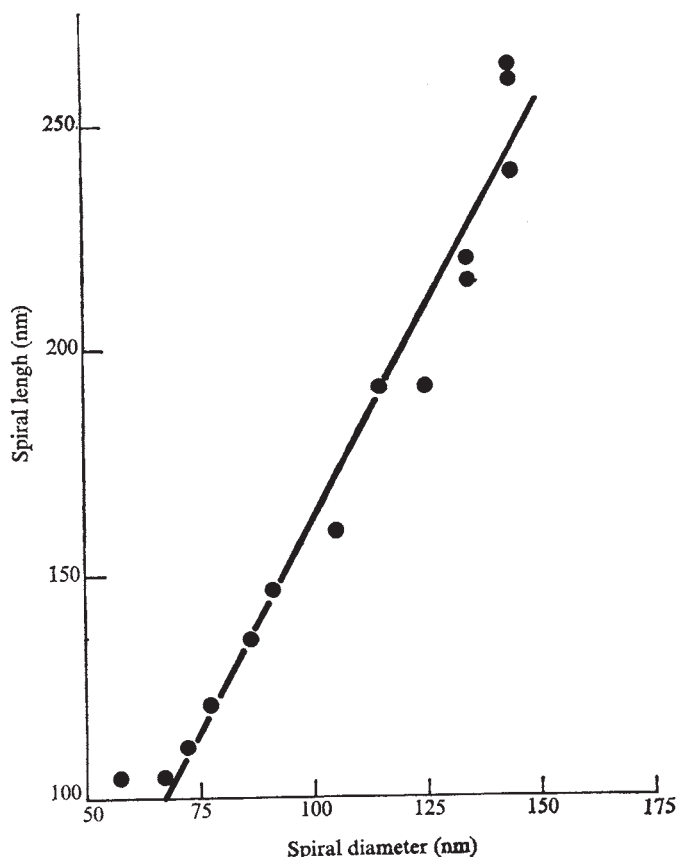


Fig. 2 Variation of spiral length with spiral diameter.

filaments and began to propagate a new pair, (Fig. 3). It is not possible at present to give a detailed account of the processes which constrain filaments to grow in this manner although it is certainly associated with the structural characteristics of the catalyst particle.

R. T. K. BAKER
P. S. HARRIS
S. TERRY

Applied Chemistry Division,
AERE Harwell,
Didcot, Oxfordshire, UK

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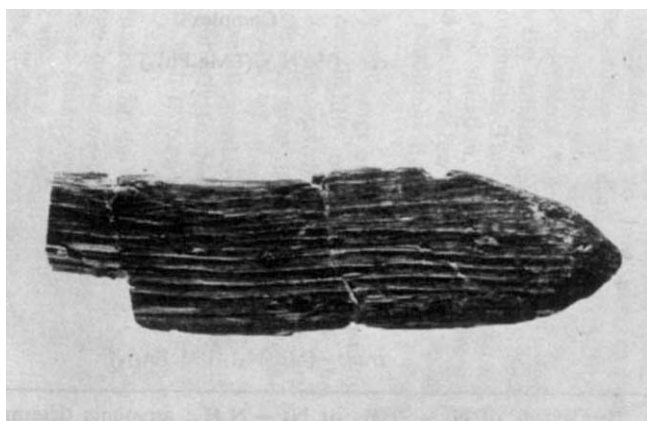


Fig. 1 This fragment of a fourth boomerang from Wyrie Swamp has been identified as *Casuarina stricta* root. Measuring 11 cm, it has been treated with polyethyleneglycol and freeze-dried.

Ancient boomerangs discovered in South Australia

DURING excavation in a South Australian peat quarry in January 1974, a wooden tool industry was found buried in basal peat formed between 10,200 $\pm$ 150 BP (ANU-1,292) and 8,990 $\pm$ 120 BP (ANU-1,293). Chert tools and chipping debris associated with swamp side encampment were also recovered from shoreline clays and underlying muds. Three implements associated with this industry are complete boomerangs (Fig. 1), suspected of being made from *Casuarina stricta* (Drooping Sheoak), a species growing above the swamp today. Although exact ages for the boomerangs are still to be determined, the finds provide the oldest evidence of the boomerang in the world and the collection as a whole is one of the most technologically complete in the Australian archaeological record. The collection of more than 25 wooden implements includes a simple short spear, at least two types of digging stick, and a barbed javelin fragment carved from a single piece of wood. Although several other implements were recovered complete, their functions are as yet unknown.

Featuring robust working edges made on large flake cores, typically worked into convex steep-edged scrapers, the stone tool industry conforms well to the 'Australian core-tool and scraper tradition' described for the 26,000-yr-old Lake Mungo industry^{1,2} and to which Pleistocene components at Kellor, Kenniff Cave, and Burrill Lake might also be assigned. Citing ethnographic examples and microscopic evidence, several researchers, particularly Jones³, have ascribed to these forms heavy wood-working tasks such as planing, cutting, debarking, and scraping—precisely the tasks Wyrie Swamp stone tools are believed to have performed. But, whereas food preparation/procurement roles are suspected of being present in the lithic technology, no direct dietary or faunal evidence was uncovered.

Current palaeobotanical studies of the peat deposits in the region made by John Dodson at the Australian National University (ANU) indicate trends in peat accumulation which appear to correlate with prehistoric utilisation of the bog. Principal Aboriginal visitation took place during an initial shallow water phase characterised by fluctuating wet, and occasional dry, episodes dated to between 10,200 and 9,000 yr ago. During this period, when the swamp was small, its shoreline became the scene of numerous tool-making and hunting and gathering activities centred on resources at the water's edge. However, 9,000 yr ago an increasingly more permanent water stand rose to cover most of the gently sloping bank, burying the shoreline debris and displacing tool-making on to the adjacent steep dune slopes. Evidence for subsequent use of the swamp appears

infrequently until 7,930 $\pm$ 160 BP (ANU-1,377), possibly in response to the relocation of aquatic plant resources.

We can therefore see the Australian Aborigine emerging from the Pleistocene equipped with a tool kit as vital to the exploitation of the local environment then as it was yesterday, and just as complex. Exactly how long this technological tradition previously existed is as yet unknown but the possibility that the boomerang soared over the shores of Lake Mungo 16,000 yr earlier seems more plausible as a result of discoveries at Wyrie Swamp.

R. A. LUEBBERS

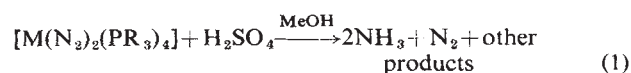
Department of Prehistory,
Australian National University,
Canberra, Australia

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The reduction of mono-coordinated molecular nitrogen to ammonia in a protic environment

WE have reduced ligating molecular nitrogen (dinitrogen) to ammonia in yields of up to 90% at a single metal site. This reaction is important for its possible application to our understanding of the chemical mechanism of the reduction of dinitrogen to ammonia by nitrogenase, where the reduction may occur at a single molybdenum ion site¹. Our reaction occurs when compounds of the type $[M(N_2)_2(PR_3)_4](I)$; $M = Mo$ or W ; $R = alkyl$ or $aryl$ are treated at room temperature with sulphuric acid in methanol solution:



This reaction was performed in a vacuum so that the evolved gases could be analysed and measured. On mixing the reagents, one molecule of nitrogen gas was rapidly evolved with a trace of dihydrogen. The remaining dinitrogen was spontaneously

Table 1 Yields of ammonia and hydrazine from reaction of H_2SO_4 with tungsten and molybdenum complexes

Complex	Solvent	% NH_3^*	% N_2H_4^*	% $\text{N}_2^\dagger$
<i>cis</i> -[W(N ₂) ₂ (PMe ₂ Ph) ₄]	MeOH	90	2	94
	MeOH	88	2	92
	MeOH/H ₂ O‡	63	3	67
	THF	46	13	59
	THF	42	23	64
<i>trans</i> -[W(N ₂) ₂ (PMePh ₂) ₄]	THF	36	15	54
	THF§	36	< 1	34
<i>cis</i> -[Mo(N ₂) ₂ (PMe ₂ Ph) ₄]	MeOH	36	< 1	40
	MeOH	30	< 1	32
	THF	20	< 1	28
	THF	35	< 1	34
	THF	4	< 1	~ 5

*Percentage of $\text{N}_2 \rightarrow 2\text{NH}_3$ or $\text{N}_2 \rightarrow \text{N}_2\text{H}_4$; ammonia determined by indophenol reagent, hydrazine by *p*-dimethylaminobenzaldehyde reagent.

† Total N_2 regained by oxidation with NaOBr of all products of reaction after Kjeldahl distillation, identity of gas confirmed by mass spectroscopy.

‡ 1 : 1 mixture by volume, dinitrogen complex in suspension.

§ Run at about -2°C , all other reactions at 20°C .

reduced to ammonia together with some hydrazine, presumably also with concomitant oxidation of the metal M.

The highest yield of ammonia (90%) was obtained from *cis*-[W(N₂)₂(PMe₂Ph)₄] in methanol. In a non-protic solvent, tetrahydrofuran (THF), the yield of hydrazine was substantially increased, but that of ammonia was halved. In aqueous methanol, the yield of ammonia was also lower, possibly due to the lack of homogeneity in the reaction mixture. The compounds examined, the effect of solvents and yields obtained are listed Table 1.

The metal-containing products from the tungsten reactions were insoluble blue solids, but with molybdenum the reaction mixture remained as a yellow-brown solution throughout. These metal products also contained some nitrogen when the solvent was THF.

We have already shown² that the ditertiary phosphine complexes, *trans*-[M(N₂)₂(Ph₂PCH₂CH₂PPh₂)₂] (II; M = Mo or W), when treated with hydrogen bromide, quantitatively form complexes of diazene, HN=NH; however, the diazene ligand could not be protonated or reduced any further in a protic solvent, except perhaps in trace amounts. Sulphuric acid has similarly given a quantitative yield of hydrazido-(2-) complexes, tentatively formulated as [M(SO₄H)(N-NH₂)(Ph₂PCH₂CH₂PPh₂)₂] SO₄H on the basis of their infrared spectra and analysis; however, no further protonation occurred to give a significant quantity of ammonia or hydrazine. Very low yields of ammonia have also been claimed from the treatment of the complex (II; M=Mo) with iron-cluster compounds, for example, [Fe₄S₄(SEt)₄]³⁻ in THF followed by acid³.

The difference between the complexes (I) and (II) which allows the reduction to proceed in the former but not in the latter, is, we believe, the ability of (I) to lose a molecule of ligating tertiary phosphine. This would allow the attack of a second sulphate (or hydrogen sulphate) ion at the metal centre, increasing its electron density. The consequent flow of electronic charge into the N₂H₂ ligand would then trigger its further protonation and reduction to hydrazine and then to ammonia.

As far as the natural system is concerned, these reactions show that molecular nitrogen can be reduced at a single metal site in a protic medium with negligible discharge of dihydrogen or displacement of dinitrogen by hydride ligands. It may also be significant, in view of the special role of molybdenum in nitrogenase, that the reduction of mono-coordinated dinitrogen has been found for the first time in molybdenum and tungsten complexes.

The most commonly proposed mechanisms of reduction involve dimetal sites^{4,5}; and by protonation of dinitrogen-

bridged dimetal complexes, yields of ammonia and/or hydrazine of up to one dinitrogen molecule reduced for each dimetal unit have been obtained^{6,7}. Our system is the first observation of reduction, in a protic medium, of one molecule of dinitrogen per transition metal atom in a well defined complex. It shows that a monometal site is at least as likely as a dimetal site in nitrogenase.

In our reduction the metal must increase its oxidation state by six units ($\text{M}^0 \rightarrow \text{M}^{\text{VI}}$), all six electrons being fed from the metal into the ligating dinitrogen molecule. This great jump in oxidation state is highly improbable in any catalytic system such as that of the enzyme, but our reaction could provide a possible model for the feed of electrons from the metal atom into the dinitrogen molecule, on the atomic level, along the atom chain M-N-N. It also suggests that such feed of electrons and consequent reduction of dinitrogen, may be triggered by replacement of some 'soft' ligand on the molybdenum by a 'hard' one such as an oxygen ligand [for example, sulphate, phosphate, hydroxide or phenoxide (from tyrosine)]; the reverse replacement would occur during the metal reduction and dinitrogen pick-up stage of the enzyme action.

J. CHATT

A. J. PEARMAN

R. L. RICHARDS

ARC Unit of Nitrogen Fixation,
University of Sussex, Falmer,
Brighton BN1 9QJ, UK

Received October 30, 1974.

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Control of aquatic weed by moth larvae

AQUATIC weeds use up nutrients in the water and so reduce the capacity of cultured areas to produce fish^{1,2}. Chemical, manual and mechanical control methods can be too

expensive³ and biological weed control is ecologically acceptable and more dependable.

The Chinese grass carp (*Ctenopharyngodon idella* Val.) has proved efficient in controlling many common aquatic weeds⁴⁻⁸. Some insects too may destroy particular aquatic weeds⁹⁻¹³ but their use for biological control has not received much attention. Philipose¹⁴ found that certain moth larvae were able to feed on the leaves of *Pistia stratiotes* L., a common noxious floating weed, but did not identify them or describe the infestation.

Here we report that the larvae of the moth, *Erastroides curvifascia* Hampson, feed specifically on foliar and stem portions of *P. stratiotes* (which is not eaten by grass carp or any other phytophagous fish) and describe their use as a potential biological control agent for the weed in fishery waters.

Field observations were made in a perennial pond (0.35 ha) with a dense infestation of *Pistia*. The numbers of larvae are high during the winter months (that is November to February) (water temperature: 22–26°C) and therefore the consumption of the host plant is also extensive during this period. The entire life cycle of the moth is completed within 27–35 d. The larval stage, which is the active feeding phase, accounts for more than 50% of the life span. The late instar larva pupate in the deep furrows of leaves and petioles of *Pistia* after feeding for about 7–9 d. The pupae metamorphose into adults in about 5–6 d.

Three sets of preliminary trials were conducted in plastic pools (capacity 500 l; surface area 6,943 m²). Field conditions were simulated by providing 50 kg of pond soil at the bottom and 200 l of pond water in each set. The weed density in the three plastic pools was identical, each receiving 1,650 g of fresh *P. stratiotes*. Two different densities of the late instar larvae, 86 m⁻² and 17 m⁻² of the surface area, were tried in the first and second pools respectively, the third being the control. The larvae in the first pool cleared 1,340 g of weed in about 30 d, and 1,230 g of weed was cleared in 40 d in the second pool. The control set recorded an increment of 960 g in weed weight at the end of 40 d reflecting the growth of *Pistia* in the absence of predation by the moth larvae. It was observed that in both the experimental sets the larvae introduced initially pupated after a brief period of feeding, resulting in the emergence of moths which immediately bred. The new early instar larvae caused most of the damage to the plants.

Our study indicates the possibility of using the larvae of *E. curvifascia* for controlling *Pista*, as they largely satisfy the basic requirements of a potential control agent. The comparative ease with which the larvae can be transferred *in situ* to host plants and the way the moths readily breed in the laboratory suggest a wide scope for disseminating this important agent for the control of *Pistia* in fishery waters.

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H. CHAUDHURI
K. JANAKI RAM

Central Inland Fisheries Research Substation,
Cuttack (Orissa), India

Received July 8; revised October 7, 1974.

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Hornet nest architecture

ALL social wasps belong to the family Vespidae. This family is divided into the following three sub-families: the Stenogastrinae—prevalent in South-east Asia, the Polistinae—of almost worldwide distribution, and the Vespinae—including the more socially developed forms which are encountered primarily in the Northern Hemisphere¹. The comb constructed by these wasps is fastened by a pedicle to the roof of the nest^{2,3}. In the course of the season the combs attain optimal size and acquire a spherical or elliptical shape, curving upwards at the centre, much like an inverted mushroom. The pedicles are long enough to allow clear passage of wasps from one comb to another.

Here I report observations and experiments carried out to elucidate the way in which hornets acquire the spatial perception necessary for the construction of combs with such clear-cut architectonic lines.

Hornets (3 d old) were maintained in groups of 30–40 individuals in specially constructed wooden breeding boxes with one wall of transparent glass⁴. Observations were made on the comb-building activities of intact hornets as compared with those of hornets with variously amputated wings. The wings were treated in one of four ways: first, one pair of wings was amputated; second, both pairs of wings were amputated; third, same as the first but subsequent maintenance of the hornets for 3–4 d at a temperature of 8°C; fourth, same as the first but with supplementation of the diet with queen pheromones (C₁₆ lactose).

The standard diet in all the experimental breeding boxes was 30% sucrose solution *ad libitum* as well as daily quotas of wasp or bee pupae. Clumps of clay soil were introduced as building material for the wasps. All experiments were carried out in a vesparium whose dimensions are given elsewhere⁵ and in which the temperature was kept at 27–28°C, as optimal for hornet activity⁶, while the relative humidity was 65–70%. Each experiment was repeated ten times.

Hornets with intact wings, after several days in the breeding box, commence building pedicles which are suspended from the roof of the box and serve as foundations for combs that architectonically resemble those produced in nature. The combs are constructed one next to the other and in parallel to the roof. The initial combs are small, each consisting of 20–30 cells, but ultimately they merge to form a single, large comb the breadth of which is functional to and only slightly smaller than the breadth of the roof. At the lateral margins of the comb there are always narrow gaps between it and the frame of the breeding box which permit movement of hornets to and from the comb. Similarly at the top of the comb, because of the pedicles which suspend the comb from the roof of the breeding box, there is a narrow space which enables passage of the worker or queen hornets as in nature. The width of this gap is 1.5–1.7 cm.

Hornets which had one pair of wings amputated also constructed a number of combs, but their construction differed in the following respects. The combs were fastened by pedicles to the wall of the breeding box rather than to its roof, that is at right angles to the norm. They were built one beneath the other, rather than one beside the other; that is, along a vertical axis rather than in the customary horizontal placement. The vertical distance between two adjacent combs was about twice that in normal or natural construction (3.2–3.4 cm). The combs do not merge but

remain discrete and small, consisting of about 50–60 cells each (Fig. 1, lower three combs). The pedicle, which in the normal comb was a compact rod narrow at the middle and wide at both ends was in this case merely a single or double row of 2–4 cells which formed a narrow bridge between the wall of the box and the comb (Fig. 1). Oviposition into the cells which substitute for a pedicle never occurred, but the workers did oviposit into other comb cells and subsequently nursed the emerging all-male brood. Structurally, the single comb of wing-amputated hornets resembled a mirror half-image of a normal complete comb.

Hornets with both pairs of wings amputated constructed their combs as described before but without a pedicle, that is, the combs were constructed right against the wall of the breeding box. Cooling or pheromone-feeding of the wing-amputated hornets caused them to revert to normal comb construction. It is worth mentioning that both hornets with

intact or amputated wings built cells of the same size and invariably the cell apertures faced downwards.

I believe that the vertical distance between two adjacent combs constructed by wing-amputated hornets should be regarded as analogous (at a 90° displacement) to the horizontal distance between two adjacent combs constructed by hornets with intact wings. The results clearly indicate that comb construction by wing-amputated hornets is different from that by normal, intact hornets. The most prominent deviation in comb construction is the different spatial orientation of the combs, which suggests that the wing tips of hornets figure importantly in the spatial perception. Amputation of the wings seemingly causes a displacement of the sensory feedback of the spatial perception and thus apparently results in deviations or errors in the orientation of construction, in incorrect distances between adjacent combs and in unilateral comb architecture. A constructing hornet, while moving about at the construction site, acquires an awareness of its spatial orientation. Von Holst⁷ designated this awareness refference, implying such impulses arise because of movement of the organism. I have no reasonable explanation as to how pheromones induce habituation of the anatomical displacement in wing-amputated hornets, but it is possible that the cooling for several days retards or prevents the ripening of their ovaries and thus inhibits the building instinct without, however, curtailing their movements. Thus the moving hornet, in its unfamiliar asymmetric situation, is able to acquire the feeling of refference and within a few days learns to orient itself to the new spatial conditions by readjustment of the sensorial feedback.

In Vespinae, the tips of the wings of the workers usually extend close to or even slightly past the tip of the abdomen. In contrast, in Stenogastrinae, the wings are always relatively short, covering only the anterior segments of the abdomen. The combs of the latter vespids are comprised of a relatively small number of cells⁸ and lack a pedicle, being fastened directly to the substrate⁹ resembling those built by hornets with two pairs of wings amputated.

It is possible that amputation of the wings of Vespinae, as I have done with *Vespa orientalis* converts the spatial perception of these hornets to that commonly displayed by Stenogastrinae. There may also be a connection between the architectural building in the Polybiine (Polistinae) wasps—phragmocytarous as against stelocytarous—and the length of their wings.

JACOB ISHAY

Department of Physiology and Pharmacology,
Sackler School of Medicine,
Tel Aviv University,
Tel Aviv, Israel

Received July 16, 1974.

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Fig. 1 Lateral view of hornet combs. The topmost comb represents normal construction. The three bottom combs were constructed by hornets who had one pair of wings amputated. The pedicle in these combs is along the same plane as the comb itself rather than vertical to it.

Extraretinal control of vertical migration in fish larvae

MANY species of fish, including the Atlantic herring *Clupea harengus* and its larvae, undergo diel vertical migrations^{1,2}. In the herring and plaice larvae this migration has been shown

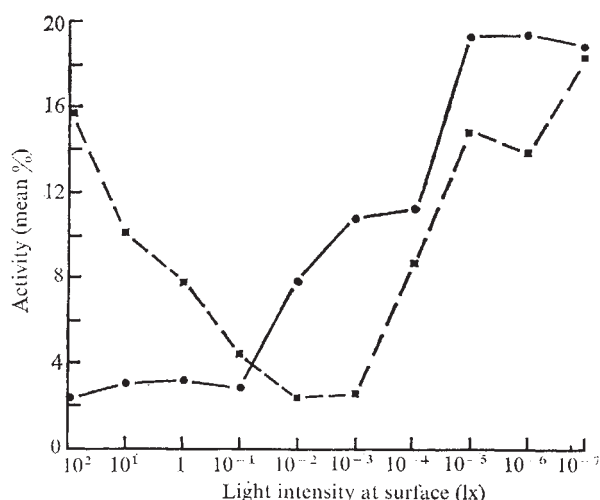


Fig. 1 The change in surface activity of normal and eyeless herring larvae in response to a reduction in ambient light intensity. Groups of 20 larvae were placed in seawater in an acrylic column (internal diameter 5.1 cm, water depth 117 cm) located in a light-tight box and illuminated from above by a 12 V 40 W tungsten lamp run at 12 V giving a light intensity of 10² lx at the water surface. The light beam passed through three glass heat filters then through one of a series of neutral density filters. The neutral density filters were located in a wheel which changed automatically every 15 min reducing the light by a factor of 10 to a final level of 10⁻⁹ times the original light intensity. A pair of matched thermistors in a balanced bridge circuit were inserted in the water column, 2 cm below the water surface, to record larval activity. Activity thus recorded has been shown to give an estimation of the number of larvae active at that level³. Larval movements detected by the cooling of the thermistors were recorded on paper for later analysis (activity only assessed during latter 10 min of exposure at each intensity). Herring and plaice larvae, both with eyes and in an eyeless condition, were tested in this apparatus. The eyes were removed surgically from anaesthetised larvae at a variety of developmental stages and as far as possible each group of experimental larvae was at the same stage. Within the range of larval stages tested the responses of the larvae were found to be comparable and the results have been pooled. The activity has been plotted as a percentage of the total activity to compensate for differences in activity level between experimental groups. The light intensity was measured with an EEL Microphotometer with a maximum sensitivity between 450 and 550 nm, the peak being about 510 nm. ●, Blind ($n = 10$); ■, controls ($n = 5$).

to be evoked by changes in light intensity³. Under laboratory conditions they move towards the water surface at dusk and return down the water column at dawn when exposed to natural light. Moreover this vertical migration can be evoked at any time of day by reducing, then increasing, the ambient light intensity³. The control of this behaviour is shown here to be at least partly mediated through extraretinal photoreceptors in the larvae of herring and plaice, *Pleuronectes platessa*.

Figure 1 clearly shows that the eyeless larvae respond to a reduction in light intensity greater than a factor of 10⁻³ by moving towards the water surface in a similar fashion to the control larvae. Also the activity, and hence the number of larvae at the surface, increases as the light intensity is further decreased. Although the activity patterns of eyeless and control larvae are not identical, they are similar enough to suggest that the same extraretinal photosense may be responsible in both conditions. Figure 2 shows that returning the light to its original intensity rapidly abolishes the surface activity of eyeless larvae, thus reversing the direction of migration.

At higher light intensities (Fig. 1) the normal larvae exhibit a high surface activity whereas the eyeless larvae remain lower in the water column. This initial response of the control animals appears to be phototactic. Blaxter⁴ has shown herring to be positively or negatively phototactic depending on the light intensity; and the range of intensities, over which this initial surface activity occurs, corresponds approximately to

that at which he observed positive phototaxis. Eyeless larvae are not phototactic over the range of light intensities 10² to 10⁻⁷ lx. Plaice larvae respond in a less marked but similar manner to herring larvae.

This correlation of the vertical migration of eyeless and control larvae is particularly interesting as it indicates that extraretinal photosensitivity plays an important role in the daily regulation of the behaviour of normal larvae. Moreover, the activity of the larvae, particularly at intermediate light intensities is influenced by the input of both the eyes and extraretinal photoreceptors. The retinal and extraretinal photosenses therefore seem to interact in producing the observed behaviour.

A suggestion that such interaction of photosenses may occur in adult fish may be seen in the work of Jones⁵. He demonstrated that minnows, *Phoxinus phoxinus*, are less active by day than by night when blind, whereas eyed fish in the absence of cover show the opposite pattern of activity. Where adequate cover is provided, however, the daytime activity of the eyed fish is considerably reduced and greater activity is displayed at night, especially observed at dawn and dusk. From this it can clearly be inferred that, as with the herring larvae, the observed behaviour results from retinally and extraretinally evoked behaviour.

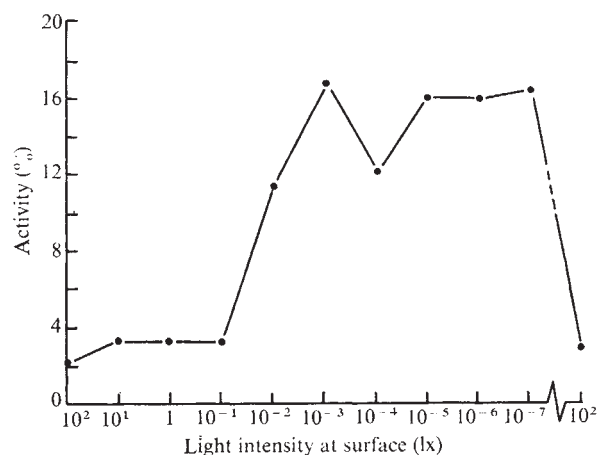


Fig. 2 The response of a single group of eyeless larvae showing the rapid manner in which surface activity is decreased when the light is returned to its original level. The interval between the increase in light intensity and activity measurement was 20 min.

It would seem that the light intensity dependent behaviour in these two examples could be divided into retinally evoked phototaxes and an extraretinally evoked kinesis which cannot be separated in sighted larvae. Further work is in progress to study the interaction of the two photosenses in modulating the animal's behaviour. Nothing can be said at this stage of the location of this extraretinal photosense but attention is drawn to a recent paper on trout⁶ which showed their diel activity pattern to be relatively unaltered by the removal of both the eyes and the pineal gland.

W. WALES

Biology Department, University of Stirling,
and Dunstaffnage Marine Research Laboratory,
Oban, Argyll, UK

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Effect of absence of cochlear outer hair cells on behavioural auditory threshold

THE function of the two populations of sensory cells in the mammalian inner ear is not well understood. Anatomical evidence indicates that the inner hair cells (IHCs) and the outer hair cells (OHCs) play separate roles in the transduction of acoustic stimuli¹. Furthermore, there have been numerous proposals attributing different roles to the two hair cell populations in the production of the various cochlear potentials²⁻⁵. On the other hand, theoretical considerations and the interpretation of data from several experiments have led to suggestions of different types of interaction between the OHCs and IHCs⁶⁻⁸.

A common means of investigating the relative roles of the two groups of sensory cells is to destroy portions of one population, usually the OHCs, and assess the changes thus produced in various indices of cochlear function. A valuable tool in such studies has been the application of aminoglycoside antibiotics. These agents are specifically toxic to cochlear hair cells, OHCs being more vulnerable than IHCs at some cochlear locations^{9,10}. Independent measurements of OHC and IHC function have been obtained by treating animals with these antibiotics, thereby largely destroying the OHC population in parts of the cochlea, while leaving much of the IHC population intact. Electrophysiological recordings from the cochlea or the VIIIth nerve were then collected and compared with responses obtained from normal inner ears³⁻⁵.

The present study was undertaken to examine the function of the OHC and IHC more directly, by measuring the behavioural pure tone thresholds of animals treated with kanamycin. We were particularly interested in determining whether or not a characteristic audiometric pattern could be related to the selective destruction of portions of the OHC population.

Eight adult male chinchillas were first trained in a shock avoidance task similar to that developed by Miller¹¹. The shuttle-box, located in a small anechoic chamber, was calibrated at 54 points spaced equally about the cage. Results were averaged to determine sound pressure levels. Stable threshold curves were usually obtained within 3-5 d after the initiation of training. One threshold curve was collected in one session per day for each animal. Ten such curves were obtained before the commencement of kanamycin treatment, and averaged to produce a baseline measure of sensitivity from 100 Hz to 16 kHz. Normal thresholds were similar to those obtained by Miller¹¹.

The animals were injected subcutaneously with kanamycin (200 mg kg⁻¹ d⁻¹). This dosage was increased to 300 mg kg⁻¹ in two animals which were resistant to the drug. Daily threshold determinations were carried out and treatment was terminated when a significant shift in threshold was observed. When the threshold shift seemed to have stabilised, ten threshold functions were again obtained and averaged to produce a post-treatment measure of sensitivity.

Animals were then anaesthetised with urethane and paired, intracochlear electrodes were implanted in scala vestibuli and scala tympani of the basal turn, following which cochlear microphonic (CM) data were collected. Finally, both cochleae were perfused with osmic acid and prepared for histological examination either by the surface preparation¹² or the Araldite embedding technique¹³.

Initial shifts in threshold occurred at the highest frequencies, appearing after 6-35 d of kanamycin treatment. The increase in threshold spread towards the lower frequencies and stabilised within 7-15 d after termination of injections. One animal became totally deaf, within the limits of our equipment to determine, but died without electrophysiological or histological data being obtained. Two animals with relatively severe hearing loss became ill and were killed without collecting electrophysiological data. The remaining five animals survived from 3 to 5 weeks after treatment was terminated, before being killed. One

animal which suffered considerable IHC damage is not included in the discussion below.

Figure 1a and b shows the OHC counts and the shifts in behavioural threshold from six animals, respectively, arranged according to the severity of hair cell loss. Note that the behavioural manifestation of OHC loss in the basal region of the cochlea was a threshold shift at high frequencies; thresholds at low frequencies remained virtually unaffected. The agreement between the extent of OHC loss and the frequency region of threshold shift is quite good. To assess this agreement, the distance from the apical end of the cochlea at which 50% OHC loss occurred, and the lowest frequency at which normal thresholds were obtained, gave a correlation (Spearman Rank Order) coefficient $r = 0.86$.

Figure 2 shows the behavioural and histological results from one animal. IHCs were present throughout the cochlea and the OHCs disappeared between 11 and 13 mm from the apex. At the higher frequencies, the threshold shift ranged from 42 to 52 dB. In the five animals surviving drug treatment, thresholds were measured at 21 shifted frequencies which were well above the transition region. The amount of shift ranged from 30.2 to 52.3 dB (mean 39.9 dB).

To ensure that the high frequency shifted thresholds appropriately reflected the response of IHCs alone, it was necessary to show that the high frequency responses were not somehow produced by the low frequency, undamaged portion of the cochlea. A masking experiment was therefore performed. White noise, low-pass filtered with a rolloff of 84 dB per octave at 3 kHz, was introduced continuously into the test environment. A noise level was chosen such that thresholds at 3 kHz and all lower frequencies were increased by about 40 dB. Figure 2b shows that the high frequency thresholds were not affected, clearly indicating that they were produced in the unmasked region of the cochlea and thus they truly reflect the sensitivity of the IHCs in the absence of OHCs. CM rolloff matched the behaviourally measured loss of sensitivity in all animals for which it was measured.

Our interpretation of these data depends on two major assumptions. First, we assume that the IHCs that appeared intact under the phase contrast microscope were functionally

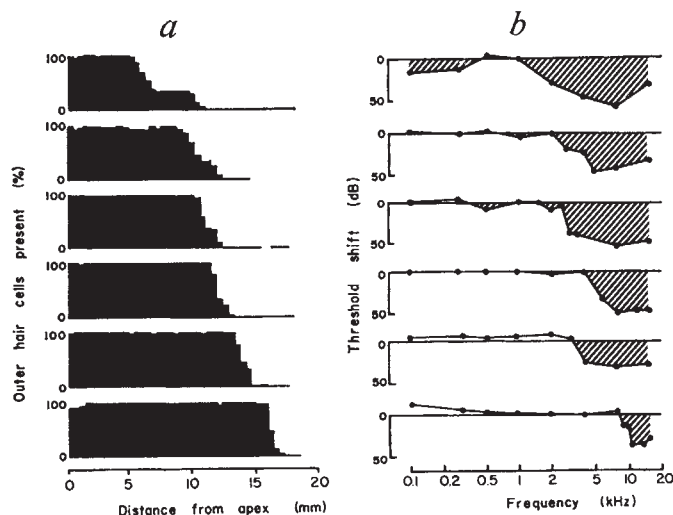


Fig. 1 a, Distribution of OHCs present in six animals, all three rows combined. Region counted is indicated by extent of the baseline. Top animal died during treatment. Ordinate, percentage of OHCs present at a given cochlear location; abscissa, distance along the basilar membrane, measured from the apical end. In all animals the IHC population (range 95-100%) was virtually intact. b, Threshold shift in the same six animals (hatching). Plots represent difference between ten audiograms obtained and averaged before and after drug treatment, except for the top animal. In the latter case, the audiogram obtained on the last day before death was used as the post-treatment measure.

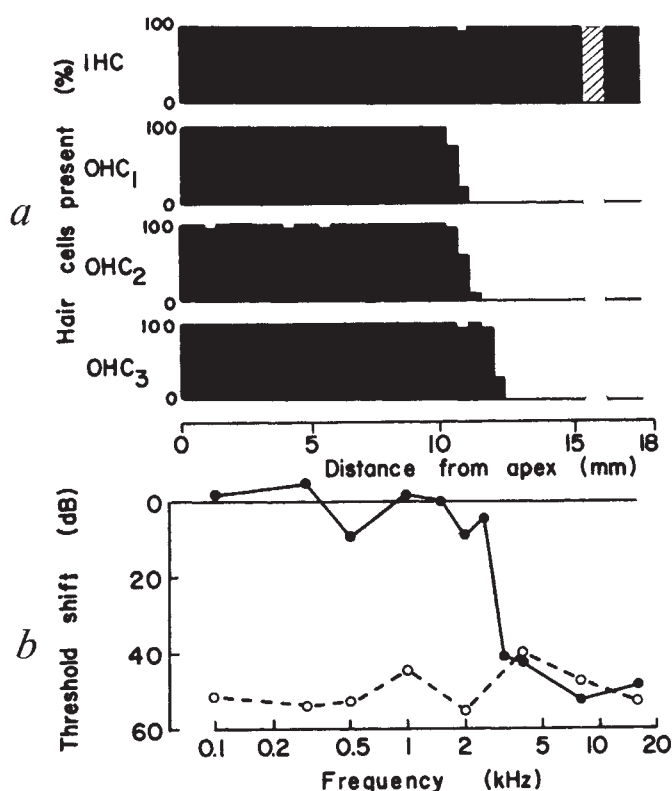


Fig. 2 *a*, Distribution of hair cells present along the cochlea in one animal. Subscript indicates row of OHCs. Hatching in the IHC plot, and gaps in the OHC baselines, represent a region of the cochlea which cannot be counted. *b*, Threshold shift measured after kanamycin treatment (●). Threshold shift measured after kanamycin treatment with low-pass (<3 kHz) masking noise present (○).

normal¹⁴. (Subtle morphological changes in hair cells, appearing normal when examined under the light microscope, may be observed with the electron microscope¹⁴.) Whether such changes influence hair cell function is open to speculation. Our working hypothesis is that subtle changes in cytoarchitecture would not significantly impair the transducer properties of the cell. While this cannot be directly confirmed, the fact that behavioural thresholds were completely normal at all frequencies below the region of threshold shift provides a basis for making this assumption. Secondly, we are assuming that the observed threshold changes are directly related to hair cell losses; that is, we assume that the drug treatment did not affect neurones of the afferent VIIIth nerve. Given the good agreement between the behavioural, physiological and histological data, this seems reasonable.

These data have several, potentially important implications. Firstly, it is apparent that in direct contrast with many studies using intense sound to create hair cell damage¹⁵⁻¹⁸, kanamycin ototoxicity yields such cochlear lesions that histological and audiometric measures are well correlated. In other words, with the well defined damage patterns observed, there is no sign of anomalous findings such as normal threshold in the presence of significant hair cell loss, or threshold shift in the absence of hair cell loss. Our data clearly show that in the chinchilla, just as in the guinea pig^{9,10}, kanamycin poisoning induces a selective OHC loss that progresses from base to apex. This permits the fashioning of cochleae in which the basal half is completely denuded of OHCs without significant IHC loss. Clearly, when one wishes to study IHC function, either with behavioural or physiological means, preparations of this type are greatly preferable to those where mixed hair cell losses tend to occur, resulting in a patchy damage pattern.

It seems therefore that the loss of OHCs has a well defined effect on auditory sensitivity. Audiometric thresholds are

about 40 dB poorer at frequencies where OHCs are absent from the corresponding cochlear location. This shift in sensitivity is extremely well correlated with the finding that the threshold change of the whole nerve action potential (AP) in guinea pigs having similar cochlear lesions is also of the order of 40 dB (ref. 5). We have also noted previously that the difference in cochlear microphonic production between OHCs and IHCs is between 30 and 40 dB (ref. 5). Thus a good three-way correlation among the various indices of cochlear function can be seen, all indicating that OHCs are the primary determinants of low intensity auditory behaviour, or at least of sensitivity. These findings are of considerable theoretical importance when one considers Spoendlin's¹⁹ demonstration that about 95% of the afferent auditory nerve fibres innervate the inner hair cells, and when the apparent homogeneity of response characteristics of auditory nerve fibres²⁰ is also taken into account. It seems probable that there is interaction between the outputs of OHCs and IHCs, either synaptic or ephaptic. Although there is considerable controversy over the existence of sufficiently close apposition (or synaptic contacts) between outer and inner hair cell dendrites to permit significant interaction^{1,21}, the experimental results seem to suggest such. These interactions do not appear to be of such nature that OHCs would inhibit IHC output as has been suggested⁸. Instead, the most parsimonious assumption is that the OHCs are specialised to perform a facilitatory function on the IHCs. The homogeneity of neural recordings suggest that all such data are probably obtained from a single population, most likely from the more numerous fibres which originate on IHCs. Behavioural and peripheral physiological data suggest that without OHCs the system is incapable of operating at low sound levels. These considerations lead to the conclusion that information processing is performed by the IHCs but that, at least at low intensities, they require facilitation by the more sensitive OHCs. Complex interactions between OHCs and IHCs have been suggested by several authors. We think that the interaction is probably not more complex than the above postulated facilitation. To demonstrate this, extensive behavioural data are necessary, collected in the absence of OHCs, on suprathreshold auditory properties and on frequency discrimination. Such data are now being obtained in our laboratory.

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ALLEN RYAN
PETER DALLOS

Auditory Physiology Laboratory (Audiology) and
Department of Electrical Engineering,
Northwestern University,
Frances Searle Building,
Evanston, Illinois 60201

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Enrichment of fungal mutants by selective cell-wall lysis

SEVERAL procedures for enrichment of fungal mutants are known and have been reviewed^{1,2} but they have many limitations. Here we describe a new technique based on selective cell-wall lysis, which has wide applicability and is highly efficient for isolating mutants differing from the wild-type strain in growth requirements.

From the time of the early basic publications^{3–7}, emphasis has been put on the importance of the physiological age of cells as regards protoplast formation by wall-lytic enzymes. Young cells are much more apt to yield protoplasts than are old ones.

Our method makes use of this fact and conditions are employed in which only the parental cells and not the desired mutants, can grow. The parental cells then become physiologically 'young' and sensitive to wall-degrading enzymes, whilst the mutants remain resistant, and can therefore be selected.

If the enzyme treatment is carried out in an osmotically-stabilised medium, the sensitive cells give rise to protoplasts which may be lysed by diluting the medium. This selective killing may also be achieved directly by using an environment not stabilised osmotically. The required mutants can then be isolated on selective media.

Here we report results obtained primarily with *Schizosaccharomyces pombe* wild-type strain L972 *h*⁺, with the adenine-requiring strain⁶ M210 *ade6 h*⁺, and with a number of auxotrophic and temperature-sensitive mutants of the wild-type strain. If not otherwise stated, cells were cultivated in liquid minimal medium containing vitamins⁷ at pH 5.5 with or without additional nutrients. The cell wall was degraded with a sterile-filtered, freeze-dried preparation of the digestive juice of the snail *Helix pomatia*. A 2% (w/v) solution of the digestive juice was prepared in this culture medium, without the use of an osmotic stabiliser. Enzyme treatment was performed with shaking at 25°C and a cell concentration of 10⁸ ml⁻¹.

To establish the optimum treatment time, suspensions in minimal medium were prepared from young and 1-week old cells of the wild-type strain. Snail enzyme preparation was added to the cells and samples were taken at intervals. Through the action of the enzyme preparation the young cells began to yield protoplasts, which immediately underwent lysis in the unstabilised medium. The rate of cell-wall degradation was followed indirectly by determination of the number of surviving cells. The data are plotted in Fig. 1a. After about 3 h practically all of the young cells had been killed, whereas the number of old cells had barely changed. On longer treatment, however, the cell-wall degradation of these also became apparent.

The behaviour of young and old adenine-requiring cells agreed with that of corresponding wild-type cells.

In the model enrichment experiments we used the wild-type strain and also the adenine-requiring mutant, which forms

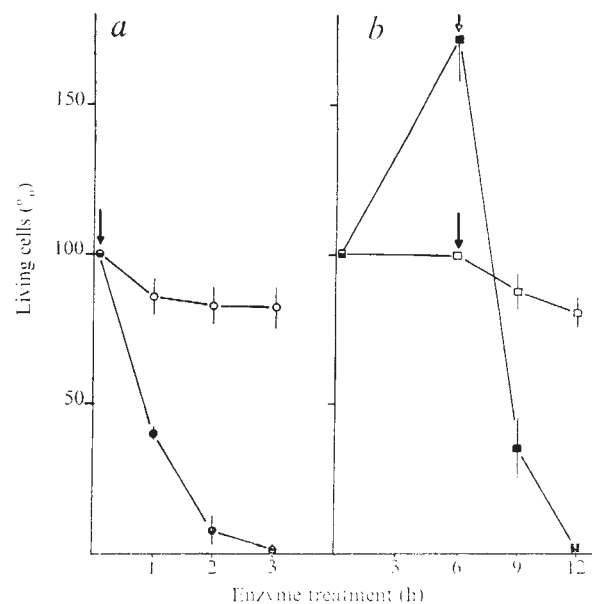


Fig. 1 Effects of enzyme treatment on *Schizosaccharomyces pombe* cells. *a*, Surviving cells of wild-type young (●) and old (○) cultures; *b*, surviving cells of wild-type (■) and adenine-requiring (□) cultures after 6 h incubation in minimal medium and 6 h enzyme treatment. Arrows indicate the addition of snail enzyme. Bars represent standard errors.

red colonies on media of low adenine content. As the colonies of the wild-type strain are white, the two types of colony can easily be distinguished. For better approximation to reality, a 100:1 mixture of wild-type and auxotrophic cells from 4-d-old colonies was used. The enzyme treatment was begun after an incubation of 6 h in minimal medium allowing exponential growth for the wild-type strain but not for the auxotrophic mutant. Figure 1b shows that after a 6 h enzyme treatment the wild-type cells were nearly all killed, while the majority of the mutant cells remained.

The efficiency of selective cell-wall lysis was enhanced markedly with the use of 2-deoxyglucose, an inhibitor of cell-wall synthesis^{8–11}. Work involving 2-deoxyglucose will be reported elsewhere. The following experiments, aimed at the isolation of new mutants, took place without 2-deoxyglucose.

In the actual enrichment process, cells of the wild-type strain, spread on the surface of yeast-extract-glucose agar medium, were subjected to ultraviolet irradiation from a Philips TUV 15 W lamp (450 erg mm⁻²; 1% survival). After subsequent incubation for 4 d, during which the mutations were manifested and the cells became old as regards cell-wall lysis, the colonies formed from the surviving cells were washed off, diluted to 10⁵ cells ml⁻¹ with minimal medium and incubated for 6 h at 25°C. This was followed by enzyme treatment for 6 h. Samples were taken before and at the end of the enzyme treatment. The samples were spread on yeast-extract-glucose agar medium, incubated for 2 d, and the colonies appearing were then replicated on to minimal medium. The mutants were isolated and their requirements determined. The degree of enrichment was found by comparison of samples taken before and after enzyme treatment.

The average values are based on data obtained with 18 different mutants, with which the ratios could be calculated exactly. In these cases the average proportion of mutants in non-enriched samples was 0.57±0.07%. The enrichment calculated on the basis of the average of the individual enrichments was 79.7-fold (*s*_i = ±14.4). No correlation could be found between the type of auxotrophic mutant and the proportion of enrichment. In many experiments it was not possible to establish the exact value of the enrichment, for the mutant could not be detected in samples which were not enriched.

Temperature-sensitive mutants from the wild-type strain have also been isolated by using ultraviolet irradiation and the enrichment procedure described above. Those mutants were isolated which could readily grow at 25°C, but for which 35°C was non-permissive. In this case an average enrichment of 80.5-fold ($s_x = \pm 17.2$) was obtained, higher even than for the nutritionally-deficient mutants.

The results can be still further improved, with correspondingly good efficiency, by repetition of the enrichment procedure.

Selective cell-wall lysis similar to that described here for *Schiz. pombe* was also attained with snail enzyme or enzymes of microbial origin for *Saccharomyces cerevisiae*, *Candida albicans*, *Geotrichum candidum*, *Rhodotorula mucilaginosa* and *Aspergillus nidulans*. Frequently, however, supplementary procedures were also necessary to achieve efficient cell-wall degradation. For *S. cerevisiae*, *C. albicans* and *G. candidum* the effect of snail enzyme was optimum in the presence of 0.1% 2-mercaptoethanol. Selective lysis could be induced in all cases with cell-wall degrading enzymes of certain *Streptomyces* and *Actinomyces*. The details will be reported elsewhere.

Protoplasts have already been produced from cells of a large number of fungal species by means of snail digestive juice or enzymes of microorganisms. Thus, the proposed enrichment method based on selective cell-wall degradation may be widely applicable to fungal mutants differing from the wild-type strain in growth requirements, and possibly to mutant cells of higher plants in cell culture.

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L. FERENCZY
M. SÍPICZKI
M. SZEGEDI

Department of Microbiology,
Attila József University,
H-6701 Szeged, PO Box 428,
Hungary

Received October 4, 1974.

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Activity of vitamin A analogues in cell cultures of mouse epidermis and organ cultures of hamster trachea

FIFTY years after the discovery that vitamin A controls cell differentiation in epithelial tissues the mechanism involved is still unknown. The recent introduction of two-cell and organ culture systems, involving serum-free medium, for the assay of vitamin A activity *in vitro* may help. One assay uses epidermal cell cultures derived from mouse skin¹ and the other uses tracheal organ cultures from vitamin A-deficient hamsters². In both, the observed cellular response depends on the addition of vitamin A, which induces a marked increase in the cellular RNA of the skin cultures and a change from the production

of keratin to the production of cilia and mucus in the tracheal organ cultures. We have now investigated the biological activity of seven analogues of natural vitamin A ester (β -retinyl acetate) and vitamin A acid (β -retinoic acid), in line with the common practice of assaying physiological and pharmacological actions of vitamins, hormones and drugs using structural analogues of a parent compound. In the analogues we used, the 5,6-cyclohexenyl ring system of natural vitamin A is modified substantially. Although data obtained *in vivo* show that modification of the ring portion of the molecule can reduce growth-promoting activity³⁻⁵, we found that several vitamin A analogues with alterations in the ring, including a shift of the 5,6-double bond of the cyclohexene ring to the 4,5-position and replacement of the cyclohexene ring with substituted aromatic and cyclopentene rings, have substantial activity in the skin and tracheobronchial assays. Because of the sensitivity of the two assays it is now possible to assay vitamin A activity in the 10^{-9} – 10^{-10} M range (30–300 pg ml⁻¹). Moreover, there is excellent correlation between the results of the two assays in terms of the evaluation of biological activity of new analogues.

Vitamin A analogues (Fig. 1) were dissolved in dimethylsulphoxide (DMSO) and stored in a liquid nitrogen refrigerator. Growth and evaluation of epidermal cell cultures¹ and tracheal organ cultures² have been described in detail. The medium for epidermal cell culture was CMRL-1066 (ref. 6), containing 0.5% crystalline bovine albumin, 2 mM glutamine, penicillin (100 U ml⁻¹), and streptomycin (100 μ g ml⁻¹); for tracheal organ culture the medium was CMRL-1066, containing crystalline bovine insulin (1.0 μ g ml⁻¹), hydrocortisone hemisuccinate (0.1 μ g ml⁻¹), and glutamine, penicillin and streptomycin as above. The final DMSO concentration in epidermal cell cultures and tracheal organ cultures did not exceed 1.0% and 0.3%, respectively; all control cultures were treated with an equivalent amount of DMSO.

The effects of each vitamin A compound on RNA, DNA and protein content of epidermal cell cultures are shown in Table 1; their activity in reversing keratinised squamous metaplasia in tracheal organ culture is shown in Table 2. Data are reported for 230 cell cultures and 563 organ cultures. There is a good concordance in the results obtained in the two assay systems. Figures 2 and 3 show the type of dose-response profile that can be obtained in the epidermal cell culture assay. It is readily apparent that the 5,6-cyclohexenyl ring of naturally occurring vitamin A can be replaced with other structures without substantial loss of activity in the two assays. However, none of the synthetic analogues used had significantly greater activity than β -retinoic acid. Two analogues,

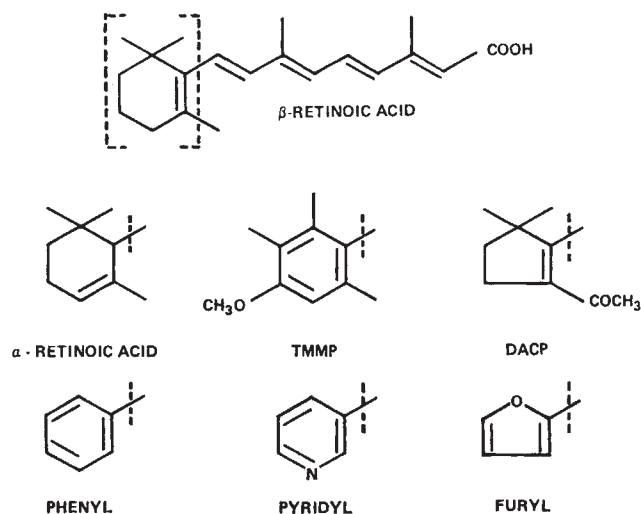


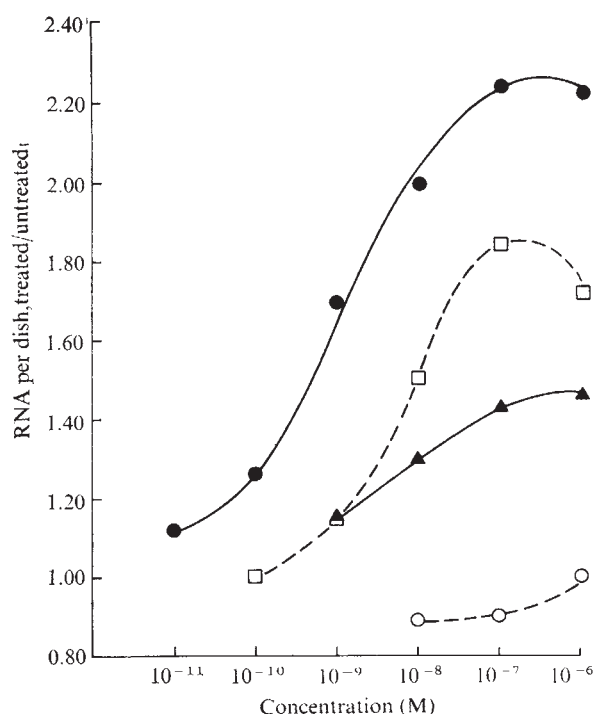
Fig. 1 Structures of β -retinoic acid and its analogues with modifications of the ring. In the analogues, the portion of the β -retinoic acid molecule shown in dotted brackets has been replaced with the structures shown.

Table 1 Percentage increase in RNA, DNA and protein in epidermal cell cultures treated with vitamin A analogues

Compound	concentration (M)	RNA	DNA	Protein
(No. of cultures)				
β-Retinoic acid				
10^{-6}	(12)	122	41	54
10^{-7}	(6)	125	46	54
10^{-8}	(12)	99	45	54
10^{-9}	(12)	70	28	44
10^{-10}	(9)	26	0	17
10^{-11}	(3)	12	-4	6
α-Retinoic acid				
10^{-6}	(6)	138	67	58
10^{-7}	(6)	96	44	26
10^{-8}	(6)	87	26	10
10^{-9}	(3)	42	0	3
10^{-10}	(3)	17	0	3
DACP analogue of retinoic acid				
10^{-6}	(6)	72	48	57
10^{-7}	(6)	84	54	69
10^{-8}	(6)	51	38	48
10^{-9}	(6)	16	21	31
10^{-10}	(3)	0	9	15
TMMP analogue of retinoic acid				
10^{-6}	(6)	148	55	33
10^{-7}	(6)	105	65	21
10^{-8}	(6)	79	51	17
10^{-9}	(3)	35	31	9
10^{-10}	(3)	26	31	9
10^{-11}	(3)	17	15	0
Phenyl analogue of retinoic acid				
10^{-6}	(9)	46	16	13
10^{-7}	(6)	44	23	20
10^{-8}	(6)	31	6	6
10^{-9}	(3)	17	—	—
Furyl analogue of retinoic acid				
10^{-6}	(6)	-1	1	14
10^{-7}	(2)	13	—	32
10^{-8}	(3)	0	—	7
Pyridyl analogue of retinoic acid				
10^{-6}	(9)	1	14	14
10^{-7}	(6)	-11	9	23
10^{-8}	(6)	-11	-6	23
β-Retinyl acetate				
10^{-6}	(6)	100	32	39
10^{-7}	(3)	116	46	44
10^{-8}	(6)	76	26	34
10^{-9}	(3)	36	5	14
10^{-10}	(3)	24	5	20
10^{-11}	(3)	12	0	9
α-Retinyl acetate				
10^{-6}	(3)	166	62	17
10^{-7}	(6)	64	21	3
10^{-8}	(6)	53	16	6
10^{-9}	(3)	12	0	3

Epidermal cells were grown for 3 d with the above vitamin A analogues. Values are expressed as the percentage increase in RNA, DNA or protein in the cultures, compared with control cultures which received no vitamin A. The mean values for such control cultures, after 3 d of growth, were as follows: total RNA per dish, 5.8 μ g; total DNA per dish, 9.3 μ g; and total protein per dish, 196 μ g. At the start of the experiment (zero-time controls), these mean values were: RNA, 5.8 μ g per dish; DNA, 7.2 μ g per dish; protein, 82.3 μ g per dish.

the furyl and pyridyl derivatives of retinoic acid, seemed to be almost devoid of activity, while the phenyl analogue had only minimal activity. There is no obligatory requirement for a 5,6-double bond in the cyclohexene ring, since both α -retinoic acid and α -retinyl acetate had marked activity in both assay systems. Similarly, both the dimethylacetylcyclopentenyl

**Fig. 2** Dose-response curves, showing effects of both active and inactive vitamin A analogues on RNA content of mouse epidermal cell cultures. Structures of analogues are shown in Fig. 1. ●, β -Retinoic acid; □, DACP; ▲, phenyl; ○, pyridyl.

(DACP) and trimethyl-methoxyphenyl (TMMP) analogues, in which the ring system has been even more substantially altered, also had marked activity in both assay systems. It is interesting that the DACP analogue of retinoic acid has been reported to block the hyperplastic and metaplastic effects of 3-methylcholanthrene on prostatic epithelium⁷, while the ethyl ester of the TMMP analogue of retinoic acid prevents skin carcinomas and papillomas in mice after exposure to polycyclic hydrocarbons⁸.

The results shown in Table 1 and Fig. 2 suggest that the action of vitamin A in the differentiation of epithelial tissues involves an effect on RNA metabolism; we have reported⁹ an abnormal electrophoretic pattern of the high molecular weight RNA molecules synthesised in tracheal epithelium of vitamin A-deficient hamsters. Effects of vitamin A in stimulating nucleoside incorporation into RNA in other tissues have also been reported¹⁰⁻¹², but in each case the molecular site of action is unclear.

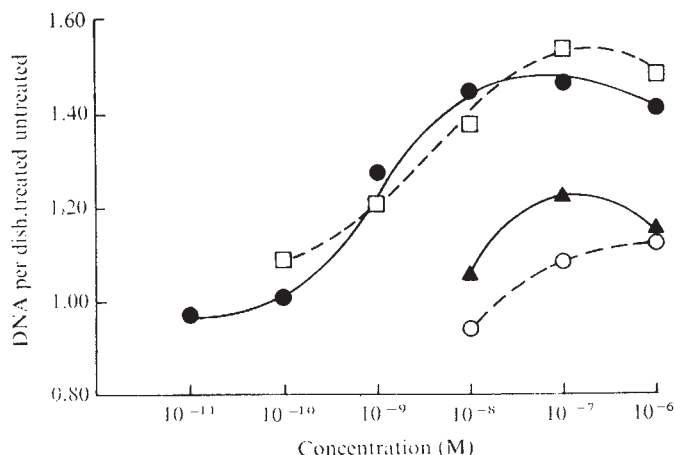
**Fig. 3** Dose-response curves, showing effects of both active and inactive vitamin A analogues on DNA content of mouse epidermal cell cultures. Structures of analogues are shown in Fig. 1. Symbols as in Fig. 2.

Table 2 Reversal of keratinised squamous metaplastic lesions of vitamin A deficiency in tracheal organ cultures treated with vitamin A analogues

Treatment of cultures (No. of cultures)		% Cultures with respective amounts of squamous metaplasia					% Cultures with keratin and keratohyaline granules
		None	Minimal	Mild	Marked	Severe	
No vitamin A, collected days 4-6	(87)	7	13	41	33	6	70
No vitamin A, collected days 11-13	(86)	2	4	33	44	17	91
β-Retinoic acid							
10^{-6} M	(46)	48	41	11	0	0	0
10^{-7} M	(22)	59	18	18	5	0	0
10^{-8} M	(19)	21	16	42	11	11	0
10^{-9} M	(11)	0	18	27	36	18	0
10^{-10} M	(12)	0	8	42	33	17	75
α-Retinoic acid							
10^{-6} M	(10)	60	30	10	0	0	0
10^{-7} M	(15)	47	13	20	13	7	0
10^{-8} M	(14)	7	7	50	36	0	0
DACP analogue of retinoic acid							
10^{-6} M	(14)	57	0	36	7	0	0
10^{-7} M	(19)	21	26	32	21	0	0
10^{-8} M	(9)	11	11	44	33	0	0
TMMP analogue of retinoic acid							
10^{-6} M	(18)	39	44	17	0	0	0
10^{-7} M	(28)	29	7	46	18	0	0
10^{-8} M	(14)	14	7	71	7	0	14
Phenyl analogue of retinoic acid							
10^{-6} M	(14)	0	21	50	14	14	50
10^{-7} M	(15)	7	13	33	27	20	67
Furyl analogue of retinoic acid							
10^{-6} M	(13)	0	8	46	38	8	77
10^{-7} M	(14)	0	14	43	29	14	79
Pyridyl analogue of retinoic acid							
10^{-6} M	(13)	0	23	61	15	0	31
10^{-7} M	(9)	0	11	33	44	11	89
β-Retinyl acetate							
10^{-6} M	(13)	61	8	23	8	0	0
10^{-7} M	(13)	15	31	31	23	0	8
10^{-8} M	(11)	9	9	64	18	0	0
α-Retinyl acetate							
10^{-7} M	(14)	21	14	36	29	0	0
10^{-8} M	(10)	0	0	10	80	10	20

All tracheas were cultured for the first 4-6 d in medium without vitamin A. At this time, some tracheas were collected, while the rest were cultured for a further week in medium containing either: no vitamin A or added vitamin A or vitamin A analogue. These tracheas were collected on the days 11-13 of culture. Cultures were graded as to the percentage of their total epithelium showing squamous metaplasia on eight cross sections from the middle of each trachea. If more than 40% of the total epithelial length was squamous, it was graded as having severe squamous metaplasia; between 10-40% was graded as marked; between 2-10% was graded as mild; and less than 2% was graded as minimal.

Obviously the *in vitro* systems for assay of vitamin A analogues do not necessarily give an evaluation of activity identical to that obtained with earlier tests of growth promotion in the whole animal. It has long been known that there may be a dissociation between the ability of a vitamin A analogue to support growth in the whole animal and to maintain specific epithelial structures, as in the case of β -retinoic acid and testicular epithelium^{13,14}. More recently, it has been shown¹⁵ that the DACP analogue of retinoic acid will not support growth in the rat, and yet we have found that this compound has definite vitamin A activity in both the epidermal and tracheal culture assays. Thus it seems that as new, more specific *in vitro* assay systems become available, the generic concept of vitamin A activity is becoming obsolete. It is increasingly essential to define the vitamin A activity of a specific chemical structure in terms of a specific biological or biochemical function, much in the same way as this was necessary some time ago for the understanding of structure-function relationships of adrenocortical steroids. The striking concordance of measured activity of vitamin A analogues in our two culture systems suggests that both systems measure a similar, fundamental biological or biochemical property of related molecules.

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MICHAEL B. SPORN
GERALD H. CLAMON
NANCY M. DUNLOP
DIANNE L. NEWTON
JOSEPH M. SMITH
UMBERTO SAFFIOTTI

Lung Cancer Branch,
Carcinogenesis Program,
National Cancer Institute,
Bethesda, Maryland 20014

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Thyroid hormone conformation from NMR studies of triiodothyropropionic acid

We have measured NMR spectra of triiodothyropropionic acid, a thyro-active analogue of the thyroid hormone triiodothyronine, to obtain information about the effects of differing solvent environments on hormone conformation.

3,5,3'-Triiodo-L-thyronine (T_3) is the most potent naturally occurring thyroid hormone, and its conformational characteristics have been much studied. The steric effects of the iodine atoms favour a conformation with the iodinated phenyl rings lying in approximately mutually-perpendicular planes, and the 120° angle at the ether oxygen produces conformational distinction between the chemically identical 3' and 5' positions on the β ring. Thus the 3'-iodine of T_3 may be positioned either proximal or distal to the α ring (Fig. 1). Although rotation of the β ring is possible, Jorgensen *et al.*^{1,2} concluded from biological testing of conformationally fixed analogues of T_3 that the 3'-iodine distal arrangement is the important one at the hormonal receptors.

An X-ray crystallographic determination of the three-dimensional structure of hydrated T_3 hydrochloride³ showed it to have the opposite conformation, that is the 3'-iodine is proximal to the α ring. Structure determinations of thyromimetic T_3 analogues, 3,5,3'-triiodothyropropionic acid ethyl ester⁴ and hydrated 3'-isopropyl-3,5'-diiodo-L-thyronine hydrochloride⁵ were performed and revealed both of these molecules also to have crystallised with conformations in which the 3'-substituent is situated in the proximal position. Subsequently, conformations with the β ring oriented with the 3'-iodine in the distal position were discovered in the crystal structure determinations of T_3 (ref. 6), 3,5,3'-triiodothyroacetic acid-N-diethanolamine⁷ and T_3 methyl ester⁷.

These results raise questions regarding the relative stabilities of the proximal and distal conformations, both in solid state and in solution, and the conditions under which each is favoured, and why. The crystals in which the 3'-substituent proximal positioning occurs were all obtained from acidic (HCl) solutions while the distal-conformation crystals were from alcohol with excesses of organic reagents often present. It seems therefore that the crystallisation medium plays a crucial role in promoting crystallisation of only one conformation or the other. Since crystallisation must be preceded by a local ordering in solution, it seemed likely to us that differences in the equilibrium populations of the proximal and distal conformers would exist in the different crystallisation solutions, and that these differences might be detectable using nuclear magnetic resonance (NMR) spectroscopy.

We chose 3,5,3'-triiodothyropropionic acid (T_3P) as a representative T_3 structural analogue for the NMR spectral study in different solutions. The solutions were chosen to duplicate as closely as possible the conditions under which proximal and distal conformations of thyromimetics were crystallised, and consisted of (i) T_3P (~ 0.08 M) in a 2 : 1 mixture of ethanol–1N HCl, the medium from which proximal

triiodothyropropionate crystallised⁴, and (ii) T_3P (~ 0.06 M), diethanolamine (~ 0.06 M), and urea (~ 0.6 M) in methanol, the relative conditions under which distal triiodothyroacetic acid-N-diethanolamine was obtained⁷. The molar concentration of T_3P used was the maximum that could be obtained under the given conditions. The spectra were recorded at room temperature on a 220 MHz spectrometer, with tetramethylsilane as an internal reference.

Figure 2 shows the NMR spectra of the aromatic-ring protons of T_3P in the two solutions. Peak assignments are labelled on the figure, and the chemical shifts are given in Table 1. The spectrum of T_3P in methanol–urea–diethanolamine is indicative of either only one species being present, or, possibly, a time-averaged distribution of species whose inter-conversion time (by β ring rotation) is very fast. The spectrum of T_3P in acidic ethanol, however, shows doubling of all the proton peaks, and is consistent with an interpretation of two distinct species being present, with a major : minor species ratio of about 3 : 1. The peaks due to the 2'-hydrogen of the major

Table 1 Chemical shifts p.p.m. of aromatic ring hydrogen atoms in T_3P NMR spectra

Atoms	CH ₃ OH–diethanolamine	CH ₃ CH ₂ OH–HCl	
	urea	Major peaks	Minor peaks
H _{6'}	6.57	6.560	6.544
H _{5'}	6.76	6.816	6.810
H _{2'}	7.01	7.000	7.028
H _{2,6}	7.81	7.760	7.775

species are displaced upfield from those of the minor component while the 5'- and 6'-proton peaks of the major species are downfield from the corresponding peaks of the minor entity; this is what would be expected if the 2'-proton of the major species were closer to the α ring and the 5'- and 6'-protons farther from the α ring than the corresponding atoms in the minor species. Thus the major entity is indicated to be the 3'-iodine proximal conformation, and the minor species the distal one. (Even the relative amounts of the displacements of the corresponding peaks of the two species agree with the relative distances of the protons from the α ring in each case.)

We also recorded a higher temperature NMR spectrum of T_3P in ethanol–HCl to observe the stabilities of the species present. No significant coalescence of the peaks occurred at approximately 60° C.

The above interpretation is consistent with and substantially clarifies the crystallographic results. In acidic (HCl) solutions the 3'-substituent proximal conformation predominates, and that is the conformation which crystallises from such media; in the other medium, from which the distal conformation crystals were obtained, only a single-species spectrum is obtained. If this represents a time-averaged distribution presumably the distal conformation is favoured (or perhaps just less soluble), but if so, the energy difference is too small for both species to be isolated by NMR at room temperature. The NMR results also pose some problems: for example (i) why does acidification of the medium cause the stabilisation of the 3'-iodine proximal arrangement, and (ii) why is the energy barrier for interconversion of the species in acidic media so high—the non-coalescence

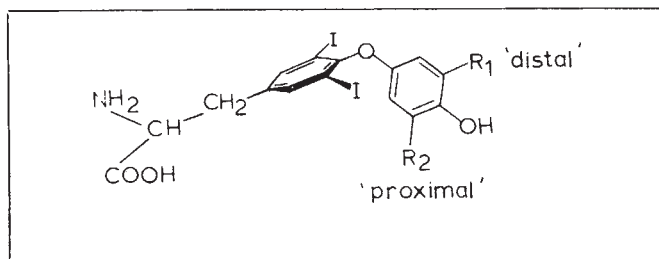


Fig. 1 Proximal and distal conformations of asymmetric β ring thyromimetics.

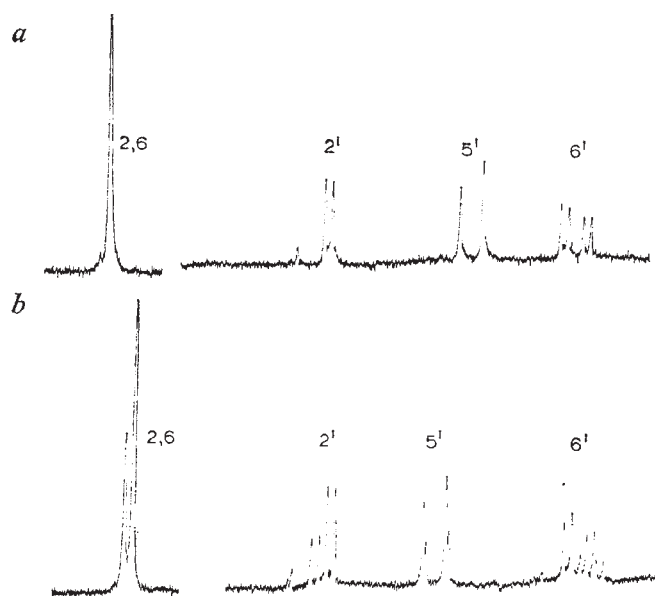


Fig. 2 NMR spectra of the aromatic ring hydrogen atoms of triiodothyronine in methanol-urea-diethanolamine (a) and ethanol-HCl (b). The field increases to the right.

of the splittings at 60° C implies a free energy of activation of at least 20 kcalorie mol⁻¹ (calculated from the 5'-hydrogen peaks separation). These questions and others pertaining to the effects of differing milieus on conformational stability are being investigated in our laboratories by systematic NMR studies at controlled pH and temperatures.

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NORMAN CAMERMAN
J. K. FAWCETT
W. F. REYNOLDS

Department of Biochemistry,
Department of Chemistry,
University of Toronto,
Toronto, Ontario, Canada

ARTHUR CAMERMAN

Departments of Neurology and Pharmacology,
University of Washington,
Seattle, Washington 98195

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Increased axoplasmic flow associated with pargyline under conditions which induce a myopathy

THE suggestion that an abnormality of the circulation might be important in the pathogenesis of Duchenne muscular dystrophy¹⁻³ has been supported by the abnormal intrafibrillar fluorescence of muscle biopsies⁴, which is presumed to be due to the presence of catecholamines⁵. Accumulation of cate-

cholamines, accompanied by the appearance of a myopathy, can be induced by the monoamine oxidase inhibitor pargyline⁵, while this myopathy can be prevented by sectioning of the sciatic nerve. This suggests that the induced myopathy is dependent on an intact nerve supply to the muscle and that catecholamines are involved in the process. Studying fast axoplasmic flow in rats treated with pargyline, we have obtained evidence of altered nerve function.

Male Sprague-Dawley rats (150-170 g) were given daily intraperitoneal injections of pargyline. The drug solution was prepared each day by dissolving 20 mg ml⁻¹ of normal saline and injecting 75 mg kg⁻¹ body weight⁶. Fast axoplasmic flow was studied in rats anaesthetised with pentobarbital (50 mg kg⁻¹) by injecting ³H-L-leucine (44.2 Ci mmol⁻¹, New England Nuclear Corp.) into the right ventral horn of the exposed spinal cord at spinal levels L-5 and L-6 as described by Lasek⁶. The animals were killed after 0.5-2.5 h, and the sciatic nerve and the L-5 and L-6 spinal roots were dissected out intact, cleaned of connective tissue and frozen immediately on a steel ruler using a Cryoquick spray. The frozen nerve was cut into 3-mm segments which were dissolved in 1 ml of Soluene 350 (Packard), 10 ml PCS (Amersham/Searle) was added and the samples were counted.

Examples of the distribution of radioactivity expressed as d.p.m. per 3-mm nerve segment as a function of the distance along the sciatic nerve are shown in Fig. 1. In the rat given saline as a control, a labelled front migrated 40 mm in 2.5 h, while 24 h after a single dose of pargyline the radioactive front extended to 35 mm within the first 1.5 h. Furthermore, as the number of doses of pargyline increased, the rate of transport of ³H increased to 52 mm h⁻¹ for rats exposed to pargyline for 3 d and 46 mm 0.5 h⁻¹ for those exposed for 7 d. For both groups the flow of material down the nerve appears to have separated into multiple fronts. This might be due to drug-induced alterations in the rate of migration of different populations of ³H-containing molecules, that is proteins and glycoproteins, both being transported by fast axoplasmic flow⁷. Slow axoplasmic

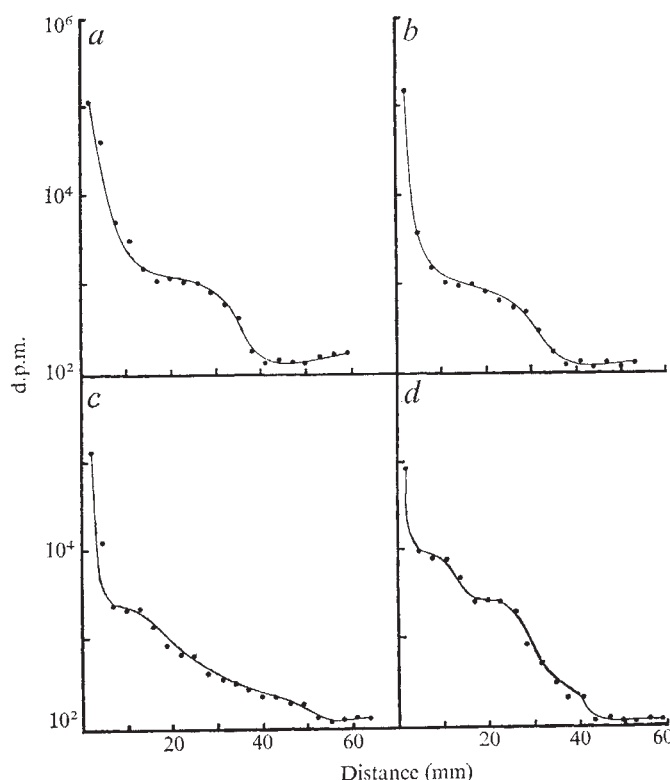


Fig. 1 Distribution of protein-bound radioactivity along the sciatic nerve of NaCl control (a) and 1 (b), 3 (c) and 7 d (d) pargyline-treated rats. Animals were killed at 2.5 h (a), 1.5 h (b), 1.0 h (c) and 0.5 h (d) respectively after labelling the spinal cord with ³H-leucine.

Table 1 Pargyline-induced increase in fast axoplasmic transport of ^3H -labelled material in the rat sciatic nerve

Pargyline 75 mg kg ⁻¹			Pargyline 25 mg kg ⁻¹		NaCl control
1 d	3 d	7 d	3 d	7 d	7 d
552±9	1,262±117	1,996±270	877±45	1,428±35	390±13
n = 4	n = 7	n = 8	n = 3	n = 4	n = 5

Figures are mm 24 h⁻¹±s.d.

flow⁸ does not appear to play a role in the development of this myopathy because the rate of slow flow is 1–2 mm d⁻¹ and necrosis is evident already after the first day of pargyline administration⁵.

The rate of migration of ^3H down the nerve was related to the number of days the rats were exposed to pargyline, increasing from 1 to 7 d, and was affected by the dose received each day (Table 1). The response elicited by pargyline could be duplicated by another monoamine oxidase inhibitor phenelzine (40 mg kg⁻¹) suggesting that an increase in monoamine levels was involved in the increase in the rate of axoplasmic flow. This was supported by our observation that pretreatment with α -methyl paratyrosine (200 mg kg⁻¹) decreases by 30% the increase in rate of fast axoplasmic flow induced by pargyline given for 3 d.

An abnormally large amount of catecholamines excreted in the urine⁹ and present in muscle⁴ of genetically dystrophic patients has been documented. In addition a model has been presented³ suggesting that a vascular abnormality with an increased sensitivity to the level of catecholamines is responsible for the development of an experimental myopathy similar to that found in Duchenne dystrophy. Monoamines are important inhibitory mediators in the central nervous system^{10,11}, and in patients with Duchenne dystrophy fewer than the usual number of functional motor units have been reported¹². We suggest that a rise in monoamine levels induced by monoamine oxidase inhibitors activates the inhibitory response in the central nervous system leading to depression of motor neurones in the spinal cord and therefore a decrease in the level of muscle activity. This could lead to an increased demand by the muscle for trophic factors which would in turn increase the rate of axoplasmic flow. A similar mechanism could explain some of the findings reported for Duchenne muscular dystrophy.

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R. J. BOEGMAN
P. L. WOOD
L. PINAUD

Department of Pharmacology,
Queen's University,
Kingston, K7L 3N6, Ontario, Canada

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New pathway for metabolism of dopa

L-DOPA (3, 4-dihydroxyphenylalanine) has, since 1967, been used in large doses clinically to lessen the degree of akinesia, rigidity and tremor in Parkinson's disease¹. The impetus for dopa therapy came from the discovery of abnormally small concentrations of dopamine in the basal ganglia of patients with Parkinsonism². Since catecholamines cannot cross the blood-brain barrier, dopa, the immediate metabolic precursor of dopamine, became the logical candidate for clinical trials. Administration of dopa increases dopamine in the basal ganglia³, and this is believed to be the primary mode of action of the drug. Although partially correct, this cannot account for the so-called on-off effect⁴—the increase in therapeutic benefits derived from prolonged use of dopa⁵—and side effects such as nausea, vomiting and personality disturbances. The latter are minimised by simultaneous administration of decarboxylase inhibitors which limit peripheral decarboxylation and allow smaller doses of dopa to be effective. Nonetheless it seems that the action of dopa is complex, with several mechanisms operative. It is therefore important to elucidate all possible metabolic fates of dopa and consider their relationship to the various effects of dopa. We report here *in vitro* evidence for a minor pathway of dopa metabolism resulting in the formation of 3,4-dihydroxyphenylacetaldehyde (DHPA) with concomitant inactivation of dopa decarboxylase.

α -Methyldopa, a poor substrate of dopa decarboxylase, inactivates this pyridoxal 5'-phosphate (PLP)-dependent enzyme⁷ as a result of abortive transamination⁸: a side reaction producing apoenzyme, pyridoxamine 5'-phosphate (PMP) and the aldehyde or ketone (in this case, 3,4-dihydroxyphenylacetone) derived from decarboxylation and transamination of the substrate. First observed in the decarboxylation of α -methylglutamate by glutamate decarboxylase⁹, abortive transamination was found to occur to a lesser degree with glutamate itself¹⁰. Mechanistically, transamination follows decarboxylation if protonation of the enzyme-bound Schiff's base occurs at C₄ instead of C _{α} (Fig. 1).

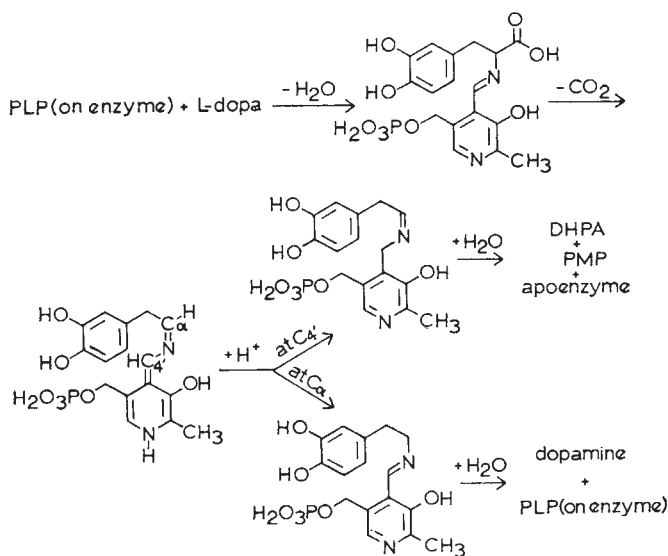


Fig. 1 Protonation of the enzyme-bound Schiff's base at C₄ and C _{α} .

Hydrolysis of the Schiff's base then yields an aldehyde or ketone and PMP, which dissociates, leaving apoenzyme. Abortive transamination may be a general reaction of PLP-dependent decarboxylases with all substrates. Because several grams of dopa per day must be metabolised by patients on dopa therapy, even a small fraction of the turnover resulting in production of DHPA and inactivation of dopa decarboxylase could be

Table 1 Ratio of radioactivity added to that isolated

Experiment	c.p.m. added as ^3H -dopa	c.p.m. isolated as 2,4-dinitrophenylhydrazones	% Transamination
1	4.7×10^7	4.1×10^4	0.009
2	4.9×10^7	6.7×10^4	0.014
3	4.6×10^7	6.2×10^4	0.013
4	4.9×10^7	6.0×10^4	0.012
			Average 0.012

Dopa decarboxylase was purified partially from hog kidney homogenate to a specific activity of $1,000 \text{ U mg}^{-1}$ (1 U = 1 nmol CO_2 produced per min) using a procedure combining aspects of published protocols^{11,12}. In a typical experiment to detect DHPA, the enzyme was incubated in 5.0 ml 0.1 M phosphate buffer, pH 7.0, containing 10^{-4} M PLP, 2 mg ^3H -dopa, synthesised¹³ by acid-catalysed exchange of aromatic protons with T_2O , in 0.4 ml buffer was added, and the reaction proceeded for 5 min. Sufficient enzyme was present to decarboxylate >90% of the substrate in this time. After addition of a small amount of unlabelled DHPA¹⁴ as a carrier, the solution was extracted with two 5-ml portions of ethyl acetate. The combined extracts were dried over Na_2SO_4 and were concentrated to approximately 50 μl with a rotary evaporator. This solution was applied to the corner of a 10×10 cm silica gel thin layer chromatography plate and was chromatographed with ethyl acetate. An ethanolic solution of 2,4-dinitrophenylhydrazine was sprayed on the spot corresponding to DHPA ($R_F = 0.54$); and a second chromatography, using ether as a solvent, was done at right angles to the original direction of elution. The orange spot ($R_F = 0.46$) corresponding to the 2,4-dinitrophenylhydrazone of DHPA was scraped from the plate and counted.

physiologically significant. We therefore investigated whether dopa decarboxylase catalyses this abortive transamination of dopa.

The detection of DHPA as a by-product of the enzymatic decarboxylation of dopa is described in the legend of Table 1. We used ^3H -dopa of high specific activity (0.1 mCi mg^{-1}) and isolated the somewhat unstable aldehyde as its 2,4-dinitrophenylhydrazone, which contained significant amounts of label. Table 1 compares the c.p.m. found in the derivative with the c.p.m. added initially as ^3H -dopa. The ratio indicates that approximately one in every 10^4 decarboxylations also results in transamination. Because of facile oxidation of the aldehyde and the semi-quantitative nature of the isolation of the phenylhydrazone, this represents a minimum value. If less enzyme was used, the radioactivity isolated was proportional to the amount of enzyme. When this experiment was done without enzyme or with heat-inactivated enzyme, less than 10% as much radioactivity was found in the phenylhydrazone. With a large excess of enzyme there was no additional radioactivity, eliminating the possibility that a trace amount of monoamine oxidase was responsible for production of DHPA.

In the absence of free PLP, dopa decarboxylase is inactivated by the abortive transamination. Enzyme freed of unbound PLP by gel filtration was incubated with 2×10^{-3} M dopa for various times. Remaining decarboxylase activity was determined by addition of 1.0 μmol of $1\text{-}^{14}\text{C}$ -dopa and collection of $^{14}\text{CO}_2$ evolved during the next 5 min. Inactivation by dopa occurred with $t_{1/2}$ of 6.6 min, comparable with the 2 min found for α -methyldopa⁸.

Addition of PLP to these partially inactivated solutions of enzyme showed that 30–50% reactivation was possible. The remainder of the original activity was lost, presumably because of the instability of the apoenzyme. There was no loss of activity when the enzyme was incubated at 37°C for 30 min in the absence of both free PLP and substrate.

The percentage of catalytic events leading to transamination can be calculated independently from the rate of inactivation. Assuming a maximum specific activity¹¹ of $9,000 \text{ U mg}^{-1}$, a molecular weight¹¹ of 112,000 and a $t_{1/2}$ for inactivation of 6.6 min, we calculate 0.01% for transamination against normal reaction—in agreement with the directly measured value.

The abortive transamination of dopa by dopa decarboxylase has been characterised by the demonstration of DHPA, inactivation of the enzyme in the absence of free PLP at a rate

commensurate with the amount of DHPA formed and the ability of PLP to reactivate the enzyme at least partially. The fractional turnover leading to transamination together with decarboxylation is small, but measurable.

Several questions can be asked concerning the importance *in vivo* of the abortive transamination of dopa. First, does DHPA arising from this pathway have physiologically significant effects which differ from those of DHPA formed by the oxidation of dopamine? Second, does the inactivation of dopa decarboxylase by this mechanism cause inhibition of dopa metabolism either peripherally or in the brain? Finally, if such inhibition indeed occurs, will the metabolism of other substrates of dopa decarboxylase be affected? The answers to these questions, when available, may aid in an understanding of dopa metabolism and Parkinsonism.

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MARION H. O'LEARY
RICHARD L. BAUGHN

Department of Chemistry,
University of Wisconsin,
Madison, Wisconsin 53706

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Histamine release by gastric stimulants

It is a matter of debate whether histamine in the gastric mucosa acts as a local chemical mediator for other gastric stimulants^{1–5}, and the isolated bullfrog mucosa provides a convenient model for investigation of the question. The preparation responds to gastric stimulants such as pentagastrin, acetylcholine and histamine; the secretory rate can be monitored easily and there are no complicating factors such as changes in blood flow. Nevertheless, conflicting reports have appeared: Davidson *et al.*⁶ found no changes in histamine content after addition of a gastrin-like pentapeptide, while Kasbekar *et al.*⁷ showed that pentagastrin and acetylcholine increased the efflux of labelled histamine. I have now used a more direct approach and found that histamine is released in response to pentagastrin and acetylcholine for which it seems to be a common mediator.

Isolated mucosa of *Rana catesbeiana* was mounted as a sheet between two sides of a leucite chamber. The serosal side had a capacity of 2.5 ml whereas the mucosal side could hold up to 4.5 ml. The normal serosal solution consisted of (mM) 89.4 NaCl, 3.0 KCl, 0.8 MgCl_2 , 18.0 NaHCO_3 , 1.8 CaCl_2 and 11.0 glucose. In the mucosal solution, glucose was omitted and NaHCO_3 was replaced by NaCl. Serosal solution was bubbled continuously with 95% O_2 /5% CO_2 and the mucosal solution

with 100% O₂. The area of the stomach exposed to the bathing solutions was 2.85 cm². The rate of secretion was monitored by a pH stat set at 7.7 as described earlier⁸. Freshly mounted stomachs secreted acid at rates varying from 6–10 $\mu\text{eq h}^{-1}$. With periodic changes of solutions, the rates of acid secretion declined during 8–12 h. Stomachs that secreted acid at rates of 2 $\mu\text{eq h}^{-1}$ or less, defined as 'resting', responded to exogenous stimulants added to the serosal solution⁹.

To estimate histamine in the serosal solution, the contents of the entire chamber were collected at various times. The starting or initial value was obtained on a sample collected in the first hour after dissection. After the stomachs had come to rest, a 30-min sample was collected ('control'). The serosal solution was then replaced with one containing one of the following stimulants: pentagastrin (5×10^{-6} M), acetylcholine (10^{-3} M) or dibutyryl cyclic AMP (5 mM). These concentrations were used to obtain an optimal increase in acid secretion. Samples were collected at 15 or 30-min intervals. After each collection, the serosal side was refilled with the solution containing the same stimulant. Aliquots (1 ml) of the serosal solution were used to estimate histamine fluorometrically with an Aminco fluorophotometer¹⁰. The method depends on the production of a fluorophore by condensation of histamine with *o*-phthalaldehyde (*o*-PT). The fluorescent and activation spectra of the fluorophore obtained with the serosal solution and with standard histamine were indistinguishable. Appropriate blanks containing the stimulants were run concurrently, these usually gave negligible results except with solutions containing dibutyryl cyclic AMP. The values of histamine obtained by these assays are expressed in terms of ng h⁻¹. This corrects for the different times of collection and also allows better correspondence with rates of acid secretion expressed as $\mu\text{eq h}^{-1}$. Freshly mounted stomachs secreted acid at a rate of $5.8 \pm 0.5 \mu\text{eq h}^{-1}$ ($n = 11$). The histamine liberated during that period was $498.1 \pm 78.8 \text{ ng h}^{-1}$. Resting stomachs secreted acid more slowly ($1.7 \pm 0.12 \mu\text{eq h}^{-1}$) and also had lower rates of histamine liberation ($135.5 \pm 12.6 \text{ ng h}^{-1}$).

Addition of pentagastrin or acetylcholine to the serosal solution led to a marked increase in the liberation of histamine and acid (Fig. 1), which reached a maximum and then declined. The release of histamine reached a peak in the first 15 min and then declined, and by the end of an hour it was near control values. In contrast, the rate of acid secretion was maximal after 25–30 min and even at the end of the hour it was significantly higher than the resting value. This suggests that the release of histamine

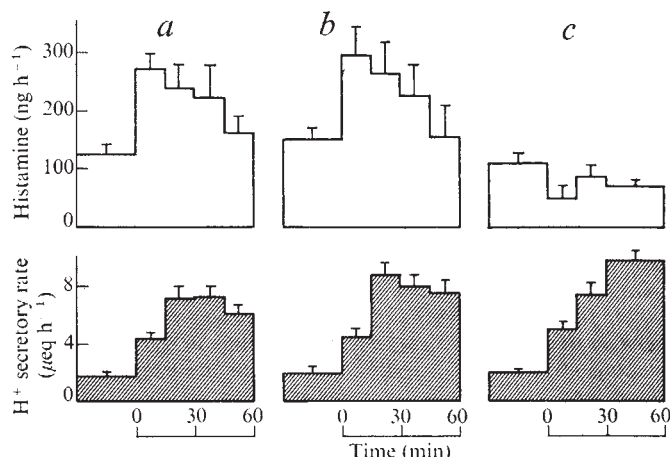


Fig. 1 Effects of adding (a) pentagastrin (5×10^{-6} M), (b) acetylcholine (10^{-3} M) or (c) dibutyryl cyclic AMP (5 mM) to a resting frog stomach are shown. The drugs were added at time zero in each case. Samples were collected at 15 min intervals in the case of pentagastrin or acetylcholine. After each collection, the serosal side was refilled with the solution containing the same stimulant. The values shown are the means $\pm$ s.e.m. for five experiments. Where dibutyryl cyclic AMP was used (four experiments), two samples were collected at 15-min intervals and the third after 30 min.

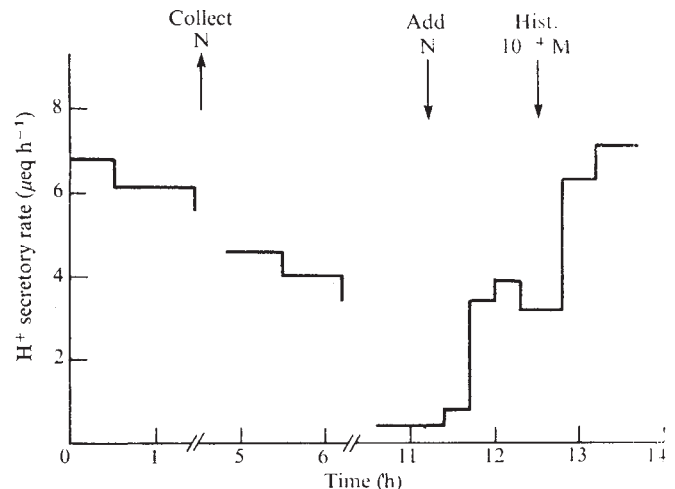


Fig. 2 Increase in acid secretion on readdition of serosal (nutrient) solution to a resting stomach. A 90-min sample was collected early in the day (collect N) when the stomach was secreting spontaneously at a high rate. The sample was stored in the cold and added back after the stomach had come to rest (add N). Sixty minutes later, 10^{-4} M histamine was added to the serosal solution.

preceded the onset of acid secretion. Dibutyryl cyclic AMP increased the rate of acid secretion but produced no significant increase in the release of histamine.

Figure 2 shows the results of an experiment in which a stomach was mounted as before, but after 30 min the serosal chamber was drained and 2.5 ml of fresh solution was added. After 90 min, the entire contents of that chamber were collected, labelled (N), and stored in the cold. After the stomach had come to rest, the serosal chamber was drained and refilled with the sample collected earlier, and allowed to warm to room temperature. As Fig. 2 shows, addition of the sample led to a marked increase in secretion of acid, which increased from $1.3 \pm 0.3 \mu\text{eq h}^{-1}$ to $3.8 \pm 0.7 \mu\text{eq h}^{-1}$ in four experiments. The stomachs also responded to exogenous histamine, 10^{-4} M. These experiments (a variation of the Loewi experiment on Vagustoff) show that the material that leaks out from the stomach into the serosal solution can stimulate acid secretion. The results of the first experiment suggest that the material is histamine.

The following conclusions can be drawn from this study. Histamine leaks from the frog gastric mucosa into the serosal solution in measurable amounts. The spontaneous secretion of the frog stomach mounted in chambers is due to histamine liberated either during pithing or dissection. The gradual decline in acid secretion presumably represents slow diffusion or metabolic degradation of the liberated histamine. The stores of histamine are not exhausted, however, and exposure to pentagastrin or acetylcholine leads to a transient release of histamine. The histamine that is released stimulates acid secretion, presumably by activating adenylcyclase¹¹. Dibutyryl cyclic AMP acting at a stage beyond that of histamine release activates acid secretion directly. Thus histamine appears to be the common mediator for acetylcholine and pentagastrin in the frog stomach.

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P. K. RANGACHARI*

Cardiovascular Research Institute,
University of California School of Medicine,
San Francisco, California 94143

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* Present address: Physiologie et Pharmacologie, INSERM U7, Hôpital Necker, 161 Rue de Sévres, 75015 Paris, France.

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Dissociation of Factor VIII-related antigen into subunits

FACTOR VIII-related antigen is present in normal human blood plasma and with conventional separation procedures it is detected in the same fractions as Factor VIII, the active blood clotting factor. Its structure and absolute concentration in plasma are unknown but it is believed to be a protein of high molecular weight. Patients with von Willebrand's disease have antigen levels and Factor VIII levels reduced in approximately the same proportions while haemophiliacs have reduced amounts of Factor VIII but normal concentrations of antigen. A differential diagnosis between these two bleeding diatheses can therefore be made by measuring antigen level. It has been suggested that the reduced amounts of Factor VIII-related antigen in von Willebrand's patients may be responsible for some aspects of the bleeding encountered with this disease and also for some of the platelet abnormalities which are found.

It has already been shown that Factor VIII readily separates into subunits under the influence of concentrated saline solution^{1,2} or reducing agents^{3,4} and the antigen remains as a large intact molecule when the Factor VIII is dissociated. This illustrates a clear distinction between the two species and a method of separating them. Here we show that it is possible to dissociate Factor VIII-related antigen and that this dissociation may be detected using either immunoelectrophoresis techniques or the 'ristocetin aggregation test'.

Human Factor VIII used in these experiments was a concentrate made according to the method described by Newman *et al.*⁵ for a material of intermediate potency. It was dissolved in 0.15 M saline to make a solution containing 10–20 units of Factor VIII activity ml⁻¹ (1 unit is defined as the amount present in 1 ml of average normal human plasma). When a sample of this starting material is subjected to gel filtration on Sepharose 4B the Factor VIII-related antigen appears in the first fraction, the so-called 'void volume'. Results of such an experiment are given in Fig. 1 where antigen concentration is plotted against volume of eluate. The column dimensions were 260 mm × 15 mm and the chromatographic procedure was identical to that described previously⁴. Antigen is measured here as a percentage of the applied sample so that a total of all plotted values equals the overall recovery which in this case is 44%. Two methods of measurement were used; one was the immunoelectrophoretic method described by Zimmerman *et al.*⁶ which is based on the Laurell technique⁷. The second method was the measurement of the effect on platelet aggregation by Ristocetin^{8,9}. It will be seen that both methods give the same results in terms of position and character of the eluted peak. Peak position is equivalent to 13.5 ml eluate which indicates a large molecular size probably greater than one million. Factor VIII itself gives a peak in the identical position⁴.

Factor VIII concentrate was reacted for 1 h at 37°C with 10⁻³ M dithiothreitol in 0.15 M saline. No buffers were

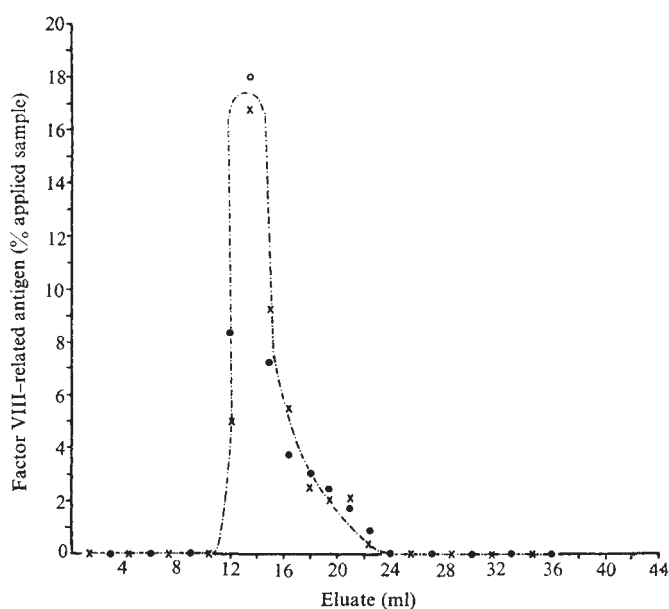


Fig. 1 Gel filtration of Factor VIII-related antigen before reaction. Antigen measured by immunoelectrophoresis (●) and ristocetin aggregate of platelets (×).

used but the solutions all had a pH between 6.5 and 7.0. After this incubation period the mixture was loaded on to a Sepharose 4B column, previously equilibrated with 10⁻³ M dithiothreitol in 0.15 M saline and the column was eluted with the same solution. In these conditions the elution pattern seen in Fig. 2 was obtained. Now the antigen appears at a point equivalent to 26 ml of eluted volume indicating that the dithiothreitol treatment has dissociated it into subunits (total recovery = 40%). Comparison with previous work⁴ shows that this elution point is the same as that obtained for Factor VIII subunits and indicates that the molecular weight of the antigen subunit is identical to that of the Factor VIII subunit at least to within ± 25%. Calibration of the column using materials of known molecular weight showed that this elution peak position was equivalent to a molecular weight of 230,000. As with the intact molecule, results obtained using the two different methods of measurement were identical.

The level of dithiothreitol used to dissociate Factor VIII-related antigen in this work was 10⁻³ M as discussed earlier. At this concentration, Factor VIII clotting activity is destroyed, most of the destruction taking place in passage of the reaction mixture through the column (which is eluted with 10⁻³ M dithiothreitol). Factor VIII itself is dissociated at 1 × 10⁻⁴ to 3 × 10⁻⁴ M dithiothreitol⁴ for the samples in question and at this concentration the Factor VIII-related antigen remains intact as a large molecule. Thus there is a very clear difference between the antigen and Factor VIII even though it appears to be one of degree. Unlike Factor VIII, it has not been possible to dissociate the antigen using high concentrations of sodium chloride but this is complicated by the fact that high saline concentrations precipitate proteins. Unlike Factor VIII it has not been possible to aggregate the antigen subunits using ε-aminocaproic acid.

There has been considerable discussion as to whether the two methods of measurement used in this work detect the same molecular species or not. As shown above these methods give identical results for the dissociated as well as for the undissociated antigen, so it must be concluded that they detect either the same or else very closely related species.

Dithiothreitol is a well known reducing agent, so it can be speculated that the subunits of Factor VIII-related anti-

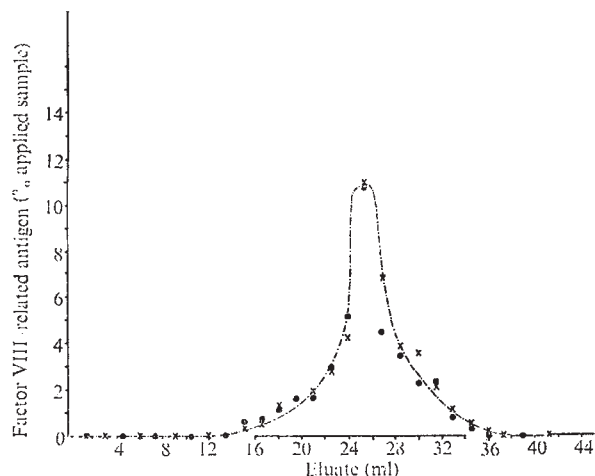


Fig. 2 Gel filtration of Factor VIII-related antigen after reaction with 10^{-3} M dithiothreitol. Antigen measured by immunoelectrophoresis (●) and ristocetin aggregation of platelets (×).

gen are joined by disulphide linkages and that these are severed by reduction in the dissociation process. A possible alternative, however, is that reduction of certain parts of the molecule have made the subunits less capable of fitting together. This possibility cannot be disregarded at the present time as such an explanation might be more acceptable in the case of Factor VIII itself when reactions other than reduction will cause dissociation.

It is becoming clear that there is an intriguing analogy between Factor VIII-related antigen and Factor VIII itself. The similarity in the size of subunits and the probable similarities in the intact molecule might suggest that they belong to the same class of compound. It could also be speculated that they each have the same number of subunits per molecule and this would be contrary to the hypothesis put forward that a single Factor VIII subunit is carried by the intact antigen. In spite of these similarities, however, there must be a distinct difference in the strength of cohesive forces binding the subunits to account for the fact that more strenuous conditions are required to dissociate the antigen. Thus, Factor VIII-related antigen and Factor VIII itself are not identical. M.A.H. acknowledges the provision of a grant by the Medical Research Council.

D. E. G. AUSTEN
M. CAREY

Haemophilia Centre,
Churchill Hospital,
Oxford

M. A. HOWARD

Department of Haematology,
Hospital for Sick Children,
London WC1N 3JH, UK

Received September 13; revised November 8, 1974.

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Modulation excitation spectro-photometry of purple membrane of *Halobacterium halobium*

WHEN *Halobacterium halobium* is grown at low oxygen concentrations in the light, it synthesises patches of membrane containing a purple pigment. If exposed to low salt concentrations, the cell membrane dissociates into fragments which differ in their protein and pigment composition and can be separated¹. The most conspicuous of these fragments is the so-called 'purple membrane'². The isolated purple membrane contains 25% lipid and 75% protein³; only a single species of protein has been found. This protein, bacteriorhodopsin, is apparently similar to the animal visual pigment; it contains 1 mol of retinal per mol protein bound as a Schiff base to a lysine residue³. The protein forms a planar lattice in the membrane⁴ and shows a broad absorption maximum at 570 nm^{3,4}. Absorption of light converts the 570 nm species to a second species which absorbs maximally at 412 nm, and in the dark reconverts to the 570 nm complex within a few milliseconds. This is apparently accompanied by a conformational change in the protein, which cycles rapidly between the two conformations, releasing protons on one side of the membrane in the first transition and taking them up on the other in the second⁵. Thus, in the intact cells the bacteriorhodopsin seems to act as a light-driven proton pump^{6,7}.

The purple membrane has been the object of several spectrophotometric studies designed to elucidate the mechanism and kinetics of the photochemical reaction. These have usually been carried out by d.c. or flash methods and often at low temperatures or in solvents chosen to arrest the decay of the photo-transients^{8,9}. Using low-temperature spectroscopy, Lozier and Stoekenius have observed four spectrally distinct intermediates analogous to the rhodopsin, prelumirhodopsin, lumirhodopsin and metarhodopsin of invertebrates⁹. The two additional forms

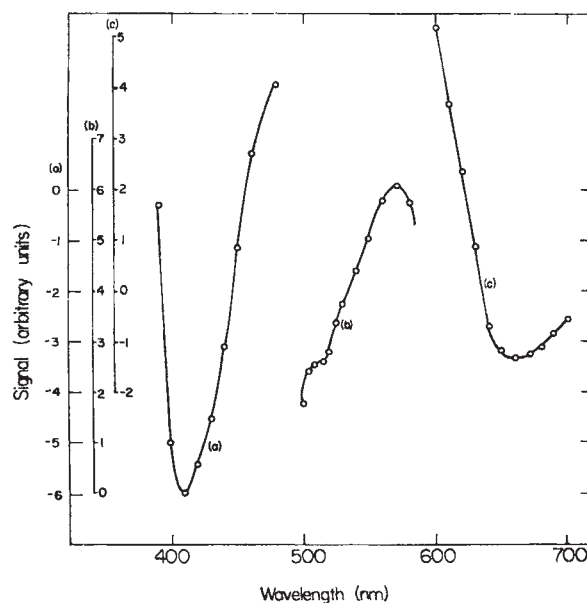


Fig. 1 Absorption spectra of transients in purple membrane suspensions in water (room temperature). Modulation frequency 5 Hz. Scales are in arbitrary units; true ratios between the peaks are $-11: +23: -3$ for 410 nm:570 nm:660 nm. Excitation was by a 250 W high-pressure mercury arc with emission extending across the ultraviolet and visible regions. Similar results were obtained by excitation at 365 nm, using a standard filter. Purple membrane suspensions were prepared from *H. halobium* R₁ as described by Danon and Stoekenius⁷; the concentration of purple complex was 0.03 mM according to A measurements based on millimolar absorption coefficient of $63 \text{ mM}^{-1} \text{ cm}^{-1}$ at 568 nm⁵. Assuming a 1:1 correspondence between the bands at 410 nm and 570 nm, their molar absorption coefficients should be in the ratio 11:23. This compares well with the ratio 11:21 for the 415 nm and 568 nm peaks according to the data of Oesterhelt and Hess⁵.

of bacteriorhodopsin were observed to have absorption bands centred at 610 nm and 550 nm, with a maximum in the difference spectrum at 510 nm. Resonance Raman spectroscopy has indicated that a Schiff-base linkage may be directly involved in the proton translocation¹⁰. Here low-temperature and solvent trapping techniques were used to study the 412 nm complex.

Modulation excitation spectrophotometry offers a convenient way of studying systems of this kind under more natural circumstances. Moreover, the light intensities used are much lower than in the case of flash photolysis. The method has been described in detail^{11,12} and briefly consists of illuminating the sample with a modulated light while at the same time observing the modulation on a constant monitoring beam, thus allowing the absorption of phototransients to be obtained. The difference of phase between the excitation modulation and that of the monitoring beam enables kinetics to be studied. Furthermore, by exciting through a polariser and analysing the monitoring beam, the polarisation of the transients may be determined.

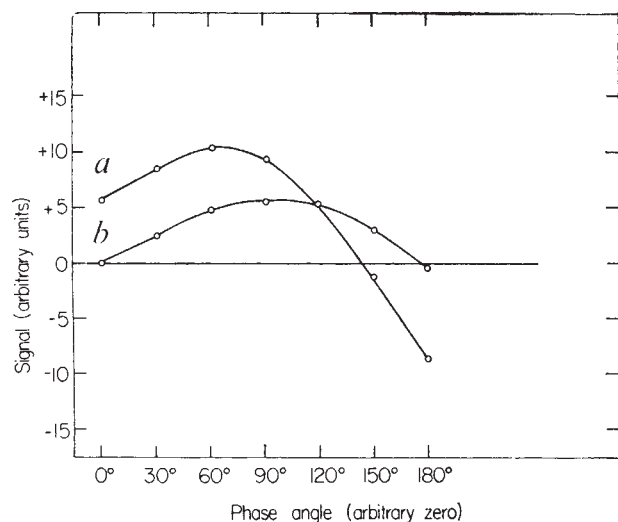


Fig. 2 The two components of polarisation at 410 nm as a function of phase angle. Modulation frequency 108 Hz. *a*, Parallel polarisation; *b*, perpendicular polarisation.

The modulation excitation spectra of a suspension of purple membrane fragments in water at room temperature are shown in Fig. 1, measured at 5 or 10 nm intervals. It can be seen that there are bands associated with the phototransients at 410 nm, 510 nm and 660 nm. In addition, the positive depletion band of the original pigments is observed at 570 nm. Although the 410 nm and 660 nm absorption bands are clearly resolved, that at 510 nm only appears as a shoulder on the depletion band. Its identity as a separate transition is established by observing the region between 500 nm and 600 nm as the phase and modulation frequency is changed, when it is observed that the shape of the absorption band in the 510 nm region changes considerably. More convincing proof comes from polarisation studies which show a sudden drop in the polarisation in the region of 510 nm, suggesting the presence of a transient in antiphase to the 570 nm transient. Moreover, if the 570 nm band is phased out to zero, the 510 nm shoulder is resolved as a distinct band.

Polarisation studies showed that the three major bands are polarised. The bands at 570 nm and 660 nm have polarisations of 0.31 and about 0.25 respectively. (Polarisation is defined here as $p = [(I_{\parallel}/I_{\perp}) - 1] / [(I_{\parallel}/I_{\perp}) + 2]$.) This suggests that the pigment is in a fairly viscous environment. The band at 410 nm at first seemed to give an anomalous result, until it was realised that the parallel and perpendicular components of the polarisation at this wavelength are not in phase, as is shown in Fig. 2. As a consequence the polarisation obtained varies with the phase setting and the modulation frequency. This indicates the presence of at least two species absorbing at 410 nm with slightly different polarisations and lifetimes.

Attempts to estimate lifetimes using the phase shift proved

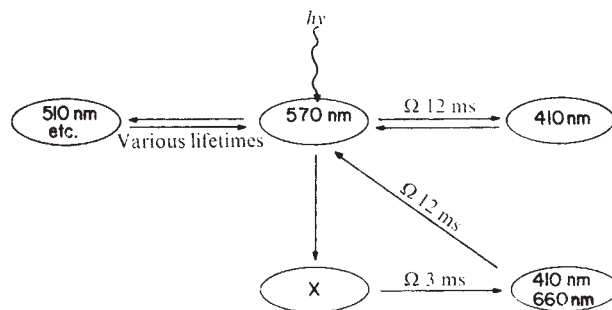


Fig. 3 Schematic diagram of kinetic processes. The two complexes absorbing at 410 nm may be conformers.

difficult since the apparent values measured varied with modulation frequency. This shows that the kinetics are not first order. The following facts have however been established. The 410 nm and 660 nm bands are in exact phase at low modulation frequency (5 Hz) with an apparent lifetime of about 15 ms, about 1 ms longer than the 570 complex. At high modulation frequency (108 Hz), the 660 nm band has an apparent lifetime of 1 or 2 ms whereas both the 570 and 410 nm bands still have long apparent lifetimes of about 12 ms, that of the 410 nm band again being slightly longer. In comparison the 510 nm transient is very short lived indeed, with a lifetime of well under 1 ms.

A flash photolysis study showed that both the depletion at 570 nm and the transient at 410 nm have lifetimes of the order of 15 ms, but the kinetics are indeed far from first order. It would appear that the depletion band also has several shorter-lived components, one major one having a lifetime of about 3 ms. The 660 nm transient passes through a well-defined cycle, rising with a lifetime of about 3 ms and decaying with a lifetime of about 12 ms.

A complete explanation of the kinetic observations is not possible at this stage, but a number of salient points emerge. First, the 410 nm band represents the primary phototransient in that the majority of molecules not in the ground state would be found in that state. Second, it seems clear that there are at least two species absorbing at 410 nm, most probably conformers with different polarisations and lifetimes. Furthermore, there is a direct kinetic link between the decay of the 660 nm transient and of the longer-lived 410 nm transient as judged from the phase relationship between them. Apparently at least three pathways exist through which the excited pigment can pass. One pathway renews the pigment rather rapidly, as shown by the observation that the phase of the 570 nm species is always slightly ahead of that of the 410 nm species at both high and low chopping frequencies; one renews it through the 410 nm transient, while the longest pathway involves the 660 nm transient. It seems certain that part of the absorption at 410 nm is associated with the transient which absorbs at 660 nm. This would account for their being in phase at the low modulation frequency. Much of the absorption at 410 nm, however, belongs to a slightly shorter-lived species or at least to a species which decays earlier than the one associated with the 660 nm absorption.

A scheme which fits these observations is shown in Fig. 3. On absorption of light by the pigment at 570 nm there is a very speedy relaxation on the part of the excitation, reforming the pigment. This is probably a trivial process of relaxation of the excited triplet-state of the original pigment. At the same time there is a quick conversion of the original pigment to the primary transient absorbing at 410 nm which relaxes slowly back to the original pigment. There is another process which over a period of about 3 ms generates the transient absorbing at 660 nm with its associated absorption at 410 nm. This also decays with a long lifetime of about 12 ms to the original pigment.

The phenomena described above were also observed in basal salt⁶, which made little difference to the spectra above 400 nm.

All samples studied were deoxygenated by purging with argon and sealing; in these conditions they were stable over periods of several weeks at room temperature. The addition of valinomycin in excess had only one effect: the 570 nm polarisation increased by about 10%, but it was found that the lifetime of this transient as measured at the 108 Hz modulation frequency decreased to a similar extent. Evidently the valinomycin quenches some of the shorter-lived species such as the triplet-state, allowing less time for rotational relaxation.

The existence of a conformational change of the 410 nm species may provide a mechanism for the proton pump, in which case the species X shown in Fig. 3 must be the original 410 nm species itself.

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MICHAEL A. SLIFKIN
S. ROY CAPLAN

Laboratory of Membranes and Bioregulation,
Weizmann Institute of Science,
Rehovot, Israel

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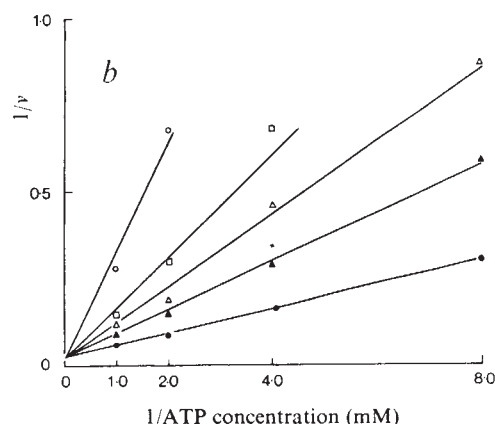
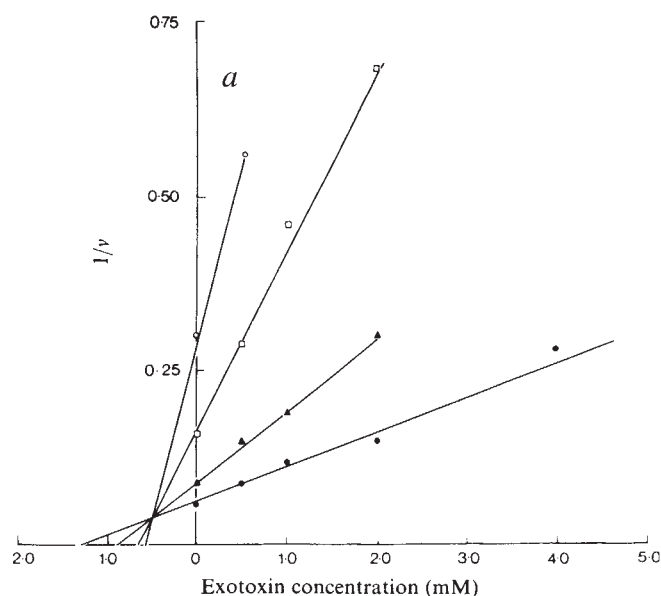


Fig. 1 Inhibition of pigeon erythrocyte adenyl cyclase by exotoxin. To combine results from several experiments, results are expressed as cyclic AMP produced as a percentage of uninhibited adenyl cyclase activity. ○, Erythrocyte 'ghosts' stimulated by sodium fluoride 1×10^{-2} M; ●, intact erythrocytes stimulated by adrenaline 1×10^{-6} M; △, unstimulated level of cyclic AMP production by 200 μ l erythrocyte 'ghosts' suspensions: mean 1.4 ng min^{-1} (range $0.6\text{--}3.0 \text{ ng min}^{-1}$); ▲, unstimulated level of cyclic AMP production by 200 μ l intact erythrocyte suspension: mean 2.2 ng min^{-1} (range $1.4\text{--}3.0 \text{ ng min}^{-1}$). Adult feral pigeons were anaesthetised with pentobarbitone (4 mg per 100 g) injected intramuscularly. Blood was drawn directly from the heart into a heparinised syringe; haemolysed 'ghosts'¹⁰ were prepared from washed erythrocytes⁸. Incubations were performed at 37° C with shaking under 95% $\text{O}_2 + 5\%$ CO_2 . The incubation mixture consisted of 200 μ l of the intact or haemolysed erythrocyte suspensions; and ATP (2×10^{-3} M) was added to 'ghost' incubations. A solution of the sodium salt of the exotoxin (5 mg ml^{-1}) was added in increasing volumes, and appropriate volumes of the modified Krebs bicarbonate buffer⁸ containing 6 mM theophylline (in which all added substances were also dissolved) were added to give a final incubation volume of 2 ml. After preincubation of the erythrocyte suspensions for 3 min with exotoxin, adrenaline (final concentration 10^{-6} M) or sodium fluoride (final concentration 10^{-2} M) was added and the incubation continued for a further 5 min. The reactions were terminated by the addition of trichloroacetic acid (TCA) to give a final concentration of 5% (w/v) and the resulting precipitate was removed by centrifugation. The TCA was extracted from the supernatant by shaking with 3 volumes of ether three times. The cyclic AMP in the supernatant was then measured by saturation analysis using the bovine adrenal cyclic AMP binding protein¹¹.

Inhibition of adenyl cyclase by an exotoxin of *Bacillus thuringiensis*

CERTAIN strains of the insect pathogen *Bacillus thuringiensis* produce a heat-stable exotoxin which is toxic to species from several orders of insects^{1,2} and also affects mammals³⁻⁵. The exotoxin inhibits ecdysone-stimulated insect RNA polymerases of mice both *in vivo*⁶ and *in vitro*⁷.

RNA polymerase catalyses nucleophilic attack by an alcohol group on the α -phosphorus of ribonucleoside triphosphates. Adenyl cyclase also belongs to this functional group of enzymes and in the studies reported here the exotoxin has been shown to be a competitive inhibitor of adenyl cyclase, to inhibit both fluoride and hormonal stimulation of this enzyme activity and to block both adrenocorticotrophic hormone (ACTH)-induced increases in adrenal cyclic adenosine monophosphate (cyclic AMP) and adrenal steroidogenesis.

The effects of exotoxin on pigeon erythrocyte adenyl cyclase were studied first. In intact avian erythrocytes, the activity of this enzyme is increased by adrenaline⁸, while in resuspended haemolysed erythrocyte preparations ('ghosts') adenyl cyclase activity is increased by sodium fluoride⁹. Figure 1 shows that the increased adenyl cyclase activity produced by sodium fluoride in 'ghosts', prepared from adult feral pigeons was markedly inhibited by exotoxin. Exotoxin also inhibited the increased adenyl

cyclase activity produced by adrenaline in intact erythrocytes, although at low exotoxin concentrations there appeared to be slightly increased cyclic AMP production.

Cyclic AMP is thought to act as the intracellular mediator of the action of many hormones¹². In the adrenal cortex, ACTH stimulates adenyl cyclase, adrenocortical cell concentrations of cyclic AMP rise; and, by a sequence of reactions not yet fully understood, increased amounts of cholesterol are converted to pregnenolone and increased steroidogenesis ensues. We have studied the effect of exotoxin on this system. Table 1 shows that ACTH produced a 38% increase in cyclic AMP content of the adrenal glands and increased 11-hydroxycorticoid production to 176% of control levels. In the presence of exotoxin, however, ACTH did not produce a significant rise in either cyclic AMP or 11-hydroxycorticoids.

To ensure that exotoxin was not interfering with corticosteroid production independently of its effects on cyclic AMP production, further incubations were performed in which, instead of adding ACTH, cyclic AMP itself was added (final concentration 5×10^{-3} M). This led to a 157% increase in 11-hydroxycorticoid production (Table 1) which was not significantly altered by exotoxin.

These results suggest that exotoxin inhibits the increased activity of adrenocortical adenyl cyclase produced by ACTH and, therefore, prevents the ACTH-induced rise in adrenocortical cyclic AMP concentrations, concomitantly reducing ACTH-stimulated steroidogenesis. Since cyclic AMP-induced steroidogenesis is not inhibited by exotoxin, other effects of the exotoxin in the mechanisms mediating steroidogenesis distant to the point of action of cyclic AMP were excluded.

The mechanism by which exotoxin inhibits adenyl cyclase activity was studied in a rat brain preparation. Figure 2 shows that exotoxin competitively inhibits adenyl cyclase in this preparation with a K_i of 0.5 mM. The K_m for ATP in this preparation was 1.1 mM.

The ability of exotoxin to compete with ATP as a substrate for adenyl cyclase is probably a property of its structural similarity to the natural substrate. Available evidence suggests that exotoxin differs from ATP in that the 5' position of ribose is substituted with a phospho-allomucyl glucoside rather than a triphosphate^{2,15}. Although a synthetic analogue of ATP, α, β -methylene ATP, has recently been shown to inhibit adenyl cyclase activity in mammalian liver and fat cell membrane preparations¹⁶, inhibition by other naturally occurring ATP analogues has not previously been reported. 3-Deoxy ATP, isolated from culture filtrates

of the moulds *Cordiceps militans*¹⁷ and *Aspergillus nidulans*¹⁸, inhibits RNA polymerase¹⁹ and seems worthy of study with respect to adenyl cyclase.

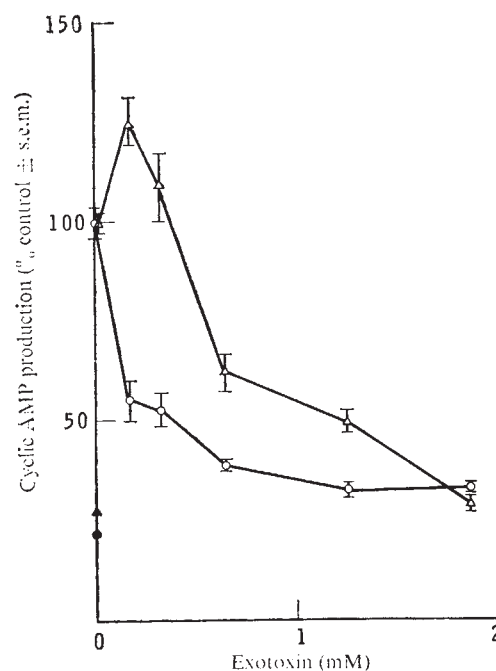


Fig. 2 Inhibition of rat brain adenyl cyclase by exotoxin. a, Effects of altering exotoxin concentration at fixed ATP concentration. ●, No exotoxin; exotoxin added: ▲, 0.5 mM; △, 1.0 mM; □, 2.0 mM; ○, 4.0 mM. b, Effects of altering ATP concentration at fixed exotoxin concentration. ATP added: ●, 1.0 mM; ▲, 0.5 mM; □, 0.25 mM; ○, 0.125 mM. v is expressed as nmol cyclic AMP produced per min by the crude mitochondrial fraction derived from 1g brain.

Male Wistar rats (200 g) were decapitated by guillotine, the brain was homogenised in 0.32 M sucrose and a crude mitochondrial fraction (P_2) was prepared¹⁴. The P_2 pellet was resuspended in 10 volumes of 0.3 M Tris-HCl buffer pH 7.4 containing 8 mM theophylline and 3 mM magnesium (v/w original brain wet weight). This suspension was then ultrasonicated for 5 min using a DAWE 1130 Soniprobe producing 75 W at 20 KHz. This mitochondrial preparation (50 μ l) was preincubated at 30°C with sodium fluoride (10 mM) in the presence of exotoxin and an appropriate volume of Tris-HCl-theophylline-magnesium buffer to give a final volume of 200 μ l. After 5 min, ATP was added and the incubation continued for a further 15 min. The reaction was stopped by boiling for 3 min, the precipitate removed by centrifugation and the cyclic AMP content of the supernatant measured.

Table 1 Effects of exotoxin on 11-hydroxycorticoid and cyclic AMP content in rat adrenal quarters*

Additions	11-Hydroxycorticoid content (μ g 100 mg ⁻¹ adrenal gland)		Cyclic AMP content (ng 100 mg ⁻¹ adrenal gland)	
	Mean	s.e.m.	Mean	s.e.m.
None	7.83	0.86	84.1	4.68
ACTH	13.75†	1.4	115.7§	5.51
ACTH + exotoxin	7.83†	0.68	86.73§	6.51
Cyclic AMP	12.28†	1.73		
Cyclic AMP + exotoxin	11.85†	1.6		

* Combined results of ten experiments; † difference significant at $P < 0.01$; ‡ difference not significant; § difference significant at $P < 0.01$.

To achieve basal levels of steroidogenesis and cyclic AMP production adult male Wistar rats (150 g) were anaesthetised with pentobarbitone (6 mg per 100 g) injected intramuscularly and decapitated by guillotine, while unconscious, 1 h later. Quartered adrenal glands were preincubated at 37°C for 90 min in Krebs-Ringer bicarbonate pH 7.4 (1.5 ml) containing glucose 100 mg per 100 ml (KRBG), under 95% $O_2 \times 5\%$ CO_2 . The glands were then removed, blotted dry, weighed and then incubated in 2.5 ml of fresh KRBG under 95% $O_2 \times 5\%$ CO_2 in the presence or absence of 6 mM exotoxin. After 15 min, ACTH (Organon) was added (final concentration 2 U ml⁻¹) and the incubation continued for 60 min. The reaction was then terminated by rapid freezing of the medium and glands in solid CO_2 and acetone. After thawing, the glands were homogenised in the incubation medium. TCA (final concentration 5% w/v) was added to 0.5 ml of the homogenate and the protein precipitate removed by centrifugation. TCA was extracted from the supernatant and the cyclic AMP measured as described in the legend to Fig. 1. In addition, 0.5 ml of the homogenate was shaken with 4 ml dichloromethane and the 11-hydroxycorticoid content of the dichloromethane extract measured by fluorimetry¹³.

Bacillus thuringiensis exotoxin appears to be a relatively specific competitive inhibitor of adenyl cyclase and may prove to be a useful tool in the investigation of the role of adenyl cyclase and cyclic AMP in various systems. Perhaps the ability of this exotoxin, and other potential adenyl cyclase inhibitors, to block a cellular physiological response to a hormone could be added to the existing criteria for the involvement of cyclic AMP in hormone actions¹².

D. G. GRAHAME-SMITH

P. ISAAC

D. J. HEAL

MRC Unit and University Department of
Clinical Pharmacology,
Radcliffe Infirmary, Oxford OX2 6HE

R. P. M. BOND

Woodstock Agricultural Research Centre,
Sittingbourne, Kent, UK

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Size and DNA content of purified *E. coli* nucleoids observed by fluorescence microscopy

PREVIOUS studies of the physico-chemical properties of DNA as isolated in condensed bacterial chromosomes suggested that the DNA was packaged in particles with dimensions similar to the intracellular nucleoid¹⁻³. This finding, if confirmed, is of significance for it indicates that current methods for isolating the condensed chromosomes preserve the conformational organisation of the DNA at least to the extent that the gross dimensions of the nucleoid are maintained.

Here we demonstrate that the isolated folded chromosomes visualised by fluorescence microscopy have dimen-

sions very similar to those of the intracellular nucleoid. This method also enabled us to determine the DNA content of the nucleoid. We found that the isolated nucleoids contain on average $15 \pm 2 \times 10^{-9}$ μ g of DNA or 3.6 ± 0.5 genome equivalents when doublet, replicating nucleoids were scored as a single structure.

The membrane-free nucleoids were isolated as previously described from *Escherichia coli* strain D-10³. The procedure for fluorescence microscopy was that of Laurent *et al.*⁴ who used ethidium bromide as a fluorescent probe to visualise kinetoplast DNA.

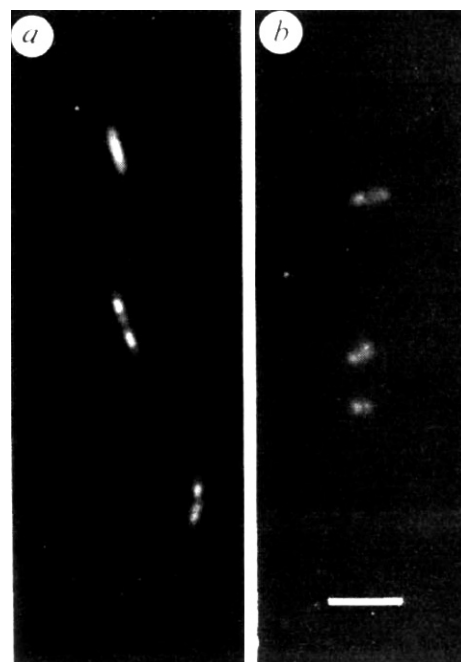


Fig. 1 Fluorescence micrographs of the nucleoid of *E. coli*. *a*, Cells in exponential growth suspended in growth medium containing $20 \mu\text{g ml}^{-1}$ ethidium bromide. The nucleoid can be seen as the brighter body occupying only part of the cell volume. *b*, Membrane-free isolated nucleoids which had been fixed with formaldehyde and suspended in a solution containing 0.01 M sodium phosphate (pH 7.8), 1.0 M NaCl, 1 mM β -mercaptoethanol, 20% sucrose, 1 mM trisodium citrate and $20 \mu\text{g ml}^{-1}$ ethidium bromide. Fixation was accomplished by mixing sucrose gradient fractions containing the isolated nucleoids with formaldehyde to a final concentration of 0.4% v/v. If Tris-EDTA buffer was present Na phosphate buffer was added to 0.05 M final concentration before the formaldehyde. After incubating for 10 min at 20°C , the fixed nucleoids were resedimented through 30% sucrose on to a self of saturated CsCl solution (45 min at 25,000 r.p.m., 4°C in an SW50.1 rotor). A Leitz Orthopan microscope with an HB 200 mercury lamp was used with a BG 12 excitation filter and a K 510 nm suppression filter. Photographs were recorded on trix-X ASA 400 film. Scale bar represents $5 \mu\text{m}$.

Figure 1a shows a fluorescence micrograph of exponentially growing cells after addition of ethidium bromide. The high spot of intensity is the nucleoid which appears to nearly fill the width of the bacterium but not its length. At the same magnification, the isolated nucleoids (Fig. 1b) appear very close in size to the intracellular nucleoid. They range in size from 0.5 to $1.5 \mu\text{m}$. Before fluorescence microscopy the isolated nucleoids were fixed by reaction with formaldehyde³. Unfixed nucleoids appeared very similar to those shown in Fig. 1; however, they were less stable to the conditions of fluorescence microscopy and they gradually expanded and became more diffuse during the microscopy (our unpublished observations). Such changes were not observed with the fixed particles. The fluorescence intensity of the isolated nucleoids is reduced relative to nucleoids

Table 1 Determination of DNA content per nucleoid

Nucleoid concentration per ml ($\times 10^{-6}$)			Concentration of radioactivity in nucleoids c.p.m. ml $^{-1}$ ($\times 10^{-5}$)	Specific Activity of DNA c.p.m. μg^{-1} ($\times 10^{-5}$)	Average μg DNA per nucleoid ($\times 10^{-9}$)		Average genome equivalents per nucleoid	
Singles (S)	Doubles (D)	Total (S + D)			S + 2D	S + D	S + 2D	S + D
24 $\pm$ 6	52 $\pm$ 4	76 $\pm$ 10	5.0	4.3	9.1 $\pm$ 1.0	15 $\pm$ 2	2.2 $\pm$ 0.2	3.6 $\pm$ 0.5
					9.4 $\pm$ 1.0	15 $\pm$ 2	2.2 $\pm$ 0.2	3.6 $\pm$ 0.5

A culture of cells (30 ml) was labelled for 20 min with 100 μCi of ^3H -thymidine (55 Ci mmol $^{-1}$) just before collection of the cells. Generation time of the cells was 29 min. Nucleoids were isolated from a portion of the culture and DNA was purified from the remainder. The method for DNA purification included lysis with lysozyme and sodium dodecyl sulphate; repeated cycles of phenol extraction; three cycles of RNase treatment interspersed with ethanol precipitations and resuspension, and passage through a BioRad P-100 column. The specific radioactivity of the DNA is listed above. The nucleoid concentration in the preparation was determined in a Petroff-Hausser chamber as described in Fig. 1. Both single (S) and double nucleoids (D) were scored. The first line of the Table shows the result of a single measurement averaged over 304 nucleoids. The second line is an average of all our data over 2,379 nucleoids. The calculated DNA content per nucleoid and the number of genome equivalents per nucleoid are tabulated above assuming, on the one hand, that there are two nucleoids per doublet and, on the other hand, that there is one nucleoid per doublet. Radioactivity was counted in a Packard liquid scintillation system; it was determined that ^3H counting efficiencies of the purified DNA and purified nucleoids were the same and hence corrections were not required.

in situ when examined as in Fig. 1. This reduction is at least partially attributable to the higher salt concentrations added to stabilise the purified nucleoids. These salt concentrations are known to increase the dissociation coefficient of ethidium bromide bound to DNA⁵.

When ethidium bromide binds to the DNA of isolated nucleoids it can affect the amount of supercoiling in the structure and thereby alter the state of condensation of the DNA^{2,7}. However, as indicated from previously published hydrodynamic properties of the nucleoids^{2,7} the conditions and concentration of ethidium bromide used in the present work should not significantly influence the final dimensions of the nucleoids. We have also demonstrated in recent work (unpublished work) that the sedimentation rate of fixed nucleoids as shown in Fig. 2 changes by less than 10% in the presence of these concentrations of ethidium bromide.

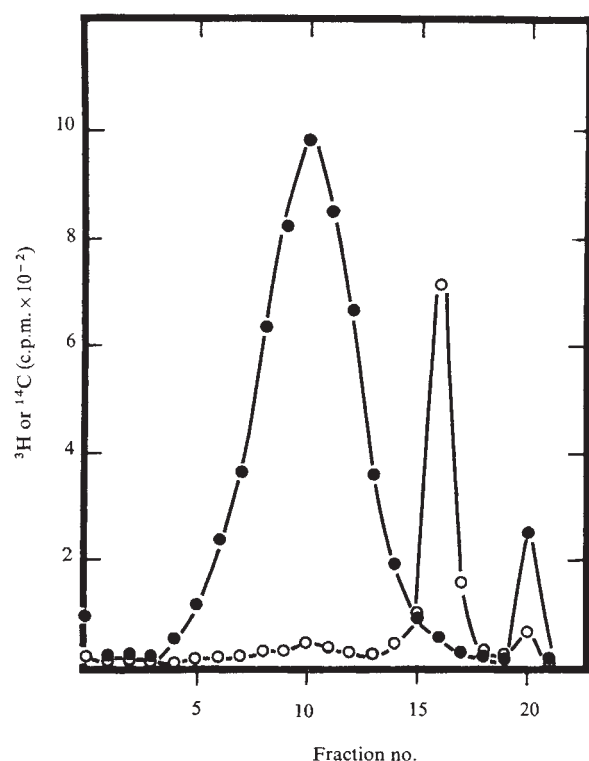


Fig. 2 Sedimentation of fixed nucleoids. After completing the observations described in Fig. 1 and Table 1, an aliquot of the same preparation of fixed nucleoids was diluted, ^{14}C -T4 phage added as a sedimentation marker (1,025S) and sedimented for 30 min at 4°C and 17,000 r.p.m. through a 10–30% w/v sucrose gradient containing 0.01 M Na phosphate (pH 7.8), 1 mM trisodium citrate and 1.0 M NaCl, $\circ$ ^{14}C -T4 phage; $\bullet$, ^3H -DNA in nucleoids.

Rapidly growing cells, such as those observed in this study, frequently contain a pair of nucleoids. When this occurs the two bodies usually seem to be separated towards the opposite poles of the cell (see ref. 8). We also observed a significant number of doublets in the preparations of purified nucleoids (Fig. 1b and Table 1). These were not generated by the fixation treatment since doubles were observed at about the same frequency in unfixed preparations of nucleoids. Resedimentation of the fixed nucleoids confirms the absence of any aggregation as well as reproducing the 1,600S average sedimentation rate of unfixed nucleoids relative to a T4 phage marker (Fig. 2). From these results, it seems likely that nucleoid pairs in the cells may actually be connected and that these connections are preserved during the isolation. Possibly the nucleoids that seem to be separated yet occur in doublets have not yet completed their division.

The concentration of nucleoids in a preparation can be measured conveniently using fluorescence microscopy techniques. From this approach we were able to determine the average DNA content of the isolated fixed nucleoids (Table 1). Depending on whether the double nucleoids were scored as a single body or two, the calculations show that on average the isolated nucleoids are made up of respectively, 3.6 or 2.2 genome equivalents of DNA. (A genome equivalent of DNA is the mass of DNA corresponding to a single, non-replicating chromosome.) When doublets are counted as a single nucleoid, the DNA content per nucleoid agrees closely with the cellular DNA content of *E. coli* cells grown under similar conditions^{9,10}. This finding offers further support to the above proposal that each of the doublets observed in our preparations was associated as a pair of nucleoids in a single cell.

In scoring the nucleoids for shape, the distinction between the doublet and singlet was not always clear. It was our impression that there may be a continuum in the degree of separation of the nucleoid halves. With this in mind it should be stressed that the distribution between doublets and singlets described in Table 1 was necessarily subjective.

After completing all of the microscopy measurements described in Fig. 1 and Table 1, an aliquot of the fixed nucleoids was resedimented on a sucrose gradient to assess the extent of unfolding or aggregation of the nucleoids which may have occurred during handling or storage. As Fig. 2 shows, the fixed nucleoids maintained their condensed state and the mean sedimentation rate of the particles was indistinguishable from the rate observed immediately after isolation^{6,7}.

In conclusion, we have shown by direct visualisation of the isolated nucleoids by fluorescence microscopy that the chromosomal DNA is isolated in a structure similar in size and DNA content to the nucleoids observed *in vivo*.

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R. M. HECHT
R. T. TAGGART
D. E. PETTIJOHN

Department of Biophysics and Genetics,
University of Colorado Medical Center,
Denver, Colorado 80220

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Polyamines stabilise DNA folds

THE DNA of bacterial cells, gently isolated in the presence of high concentrations of monovalent salts, remains folded in a particle resembling the bacterial nucleoid^{1,2}. The double-helical DNA is condensed by folding¹ and by supercoiling³ and the compact structure is dependent both on counterions in the solvent environment and RNA molecules bound to the DNA¹⁻⁴. In the absence of these stabilising interactions the DNA unfolds and acquires the usual properties of extended

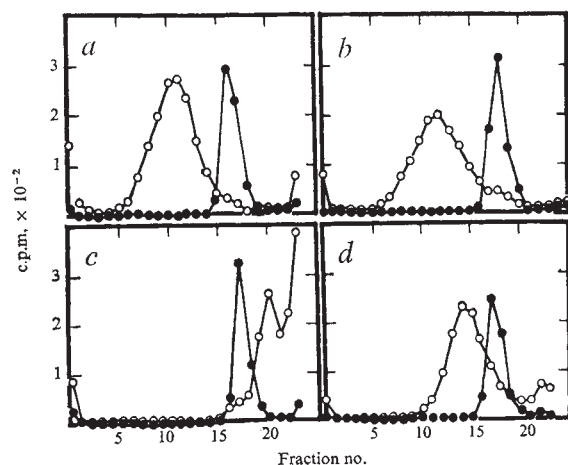


Fig. 1 Sedimentation of folded and unfolded DNA after various periods of storage. Membrane-free nucleoids labelled with ³H-thymidine were isolated on sucrose gradients containing 1.0 M NaCl and other components as described in text; peak fractions were pooled and half of the pool was stored at 4° C. The other half was also stored at 4° C but in the presence of 5 mM spermidine and with NaCl diluted to 0.25 M while holding other components of the gradient buffer constant. At various times aliquots were removed, diluted to approximately the same DNA concentration (0.5-1.5 µg ml⁻¹) and ¹⁴C-labelled T4 phage (1,025S, ref. 7) were added as a sedimentation marker. The mixture was layered on a 4.8 ml sucrose gradient (10-30% w/v) containing 0.4 M NaCl, 1.0 mM spermidine and other components as described in the text and centrifuged at 17,000 r.p.m. at 4° C for 30 min in a SW50.1 rotor. The DNA was stored in 1.0 M NaCl for 1 h (a) or 6 d (b) in 0.25 M NaCl plus 5 mM spermidine for 1 h (c) or 6 d (d). ○, ³H-DNA; ●, ¹⁴C-T4 phage.

double-helical DNA^{1,3}. The concentrations of monovalent salts required during the isolation of folded DNA are significantly greater than those found in the cell. It is, however, believed that *in vivo* polyamines may be an important counterion neutralising DNA⁵. We have now found that moderate concentrations of the polyamine spermidine greatly stabilise the condensed DNA conformations in isolated nucleoids. In addition to broadening current understanding of the role of counterions in stabilising condensed DNA states, this finding will be of significance to studies in which the stability of the nucleoid *in vitro* is important.

Nucleoids were isolated from *E. coli* strain D-10 as before^{1,2}. We used the membrane-free nucleoid² (1,600S), obtained by incubating the lysate before DNA isolation at 20° C in the presence of 0.5% Brij 58-0.2% deoxycholate. The folded DNA of this particle has less than 10% by weight bound protein, most of which is DNA-dependent RNA polymerase^{1,2,6}. Nascent RNA chains are also bound to the folded DNA; the RNA fraction is 0.4 relative to DNA (w/w) (ref. 2). In some cases purification was on sucrose gradients containing 0.01 M Tris (pH 8.1), 1 mM EDTA, 1 mM β-mercaptoethanol and 1.0 M NaCl; in other cases, the gradients contained the same mixture, but with 0.4 M NaCl and 1 mM spermidine. The results described here were similar using both kinds of preparations.

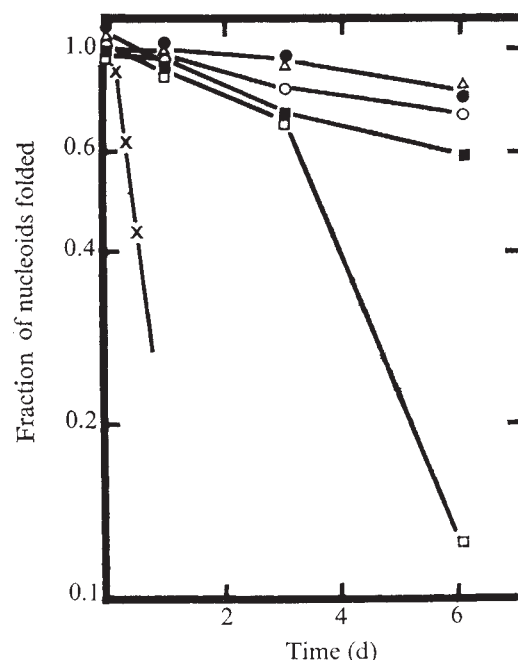


Fig. 2 Stabilisation of folded DNA with spermidine. Isolated nucleoids were stored at 4° C in the presence of varied amounts of spermidine and at times later were sedimented as described in Fig. 1 to estimate the level of unfolding (see text for definition of 'folded'). □, 1.0 M NaCl minus spermidine; ×, 0.10 M NaCl minus spermidine; other points had 0.25 M NaCl plus spermidine: 10 mM (●); 5 mM (△); 2 mM (○); or 1 mM (■).

As Fig. 1a shows, the folded DNA immediately after isolation sedimented at about 1,600S in a band somewhat broader than that of a homogeneous molecular weight marker. Worcel and Burgi³ have demonstrated that the slight heterogeneity is attributable to the variation in DNA content of nucleoids in different stages of replication. During prolonged storage, with monovalent salts as the stabilising counterions, the DNA gradually unfolded and we observed transition to slower sedimenting structures (Fig. 1c). The evidence that the sedimentation change was derived primarily from conformational alteration of the DNA rather than a reduction of the mass or molecular weight of the structure is essentially the same as reported¹⁻⁴. As in the case of the transition resulting from

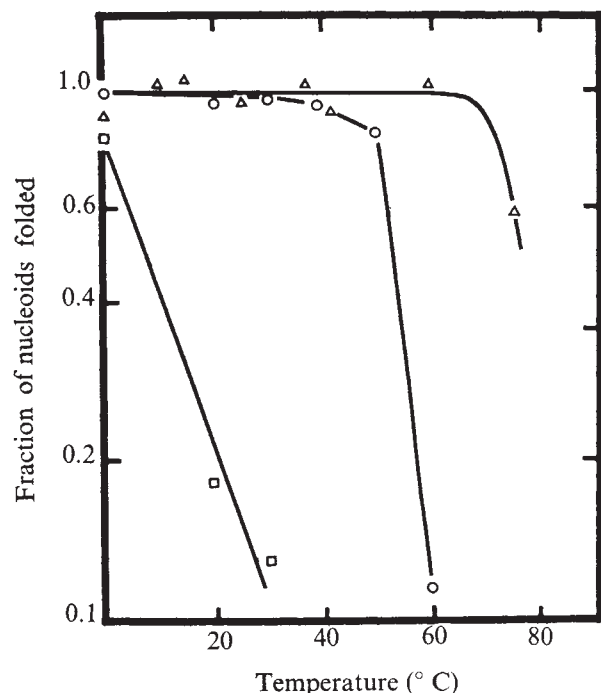


Fig. 3 Thermally induced unfolding in the presence or absence of spermidine. Isolated nucleoids were diluted to the same DNA concentration in different solutions so that all components of the storage buffer remained constant (see Fig. 1) but the NaCl concentration became 0.1 M and the spermidine concentration was 5 mM (Δ) and 2 mM ($\circ$) or none was added ($\square$). The solutions were heated for 5 min at the temperatures shown, chilled to 0°C and sedimented as described in Fig. 1 to estimate the level of unfolding.

treatment with reagents which unfold the DNA, there was a simultaneous large increase in relative viscosity and reduction in sedimentation rate. Also, since the mass of the isolated nucleoid is due predominantly to DNA, any dissociation of RNA or protein should by itself not have led to a large reduction in sedimentation rate. Furthermore, our preliminary sedimentation studies (unpublished) of DNA obtained from partially unfolded nucleoids indicate no large change in DNA molecular weight.

To describe the changes that occur in data such as those of Fig. 1, we use the following rough criterion. Folded DNA is said to have been unfolded or partially unfolded if, without breaking the DNA, it undergoes transition from a structure sedimenting more rapidly than 1,000S to one sedimenting at less than 1,000S. Until there is a more quantitative understanding of unfolding this criterion will suffice for approximation.

The rate of DNA unfolding during storage was reduced in the presence of spermidine (Fig. 1). As Fig. 2 shows, unfolding rate was minimal with 5 or 10 mM spermidine and increased with lower concentrations. In the absence of spermidine the nucleoids were not stable for more than a few days; however, they were much more stable in 1.0 M than in 0.1 M NaCl.

Spermidine also stabilised against thermally induced unfolding (Fig. 3). In a solvent containing 0.1 M NaCl and 2 mM spermidine the temperature at which half of the nucleoids were partially unfolded appears to be approximately 55°C measured under the conditions described in Fig. 3, while it increased to more than 70°C in the presence of 5 mM spermidine. In the same solvent without spermidine the analogous temperature was about 15°C and nearly all the DNA was partially unfolded at 37°C (see Fig. 4 for relevant sedimentation profiles). The sharpness of the thermally induced transition suggests that the molecular interactions stabilising folded DNA, like those stabilising the double helix, are cooperative. By comparison the T_M for denaturation of double helical *Escherichia coli* DNA in the same solvent without

spermidine was 85°C and with 5 mM spermidine added it increased to about 90°C.

The sedimentation method used here to observe the unfolding transition, is particularly sensitive to partial unfolding of DNA⁴. Using the criterion described above, DNA which has partially unfolded and sediments at rates less than 1,000S is scored the same as completely unfolded DNA. One would therefore expect that when precise methods are available for quantitating the amount of unfolding, the transition temperatures required to remove half of the DNA folds will be somewhat larger than our estimates.

We occasionally observed an apparent aggregation of isolated nucleoids mediated by spermidine. This was most obvious at the highest spermidine concentrations (above 2 mM) and at the lowest DNA concentrations (less than 1 μ g DNA ml⁻¹). It was manifested by an increased sedimentation rate and increasingly broad sedimentation profile of the nucleoids. In extreme cases at high spermidine concentrations complete precipitation of the nucleoids was observed using the sedimentation conditions described in Fig. 1.

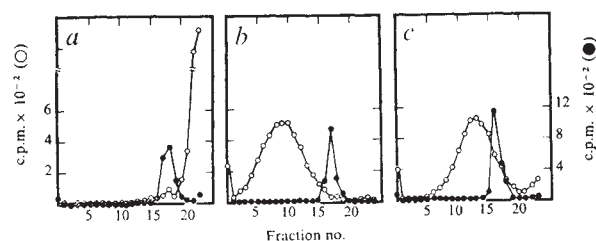


Fig. 4 Sedimentation profiles of folded DNA after heating. Three sucrose gradients from the data summarised in Fig. 3 are shown: (a) 37°C minus spermidine; (b) 37°C, 2 mM spermidine; (c) 60°C, 5 mM spermidine. $\circ$, ³H-DNA; $\bullet$, ¹⁴C-T4 phage.

Polyamines are known to stabilise different nucleic acid structures and protein-nucleic acid complexes. Examples are such interactions with double-helical DNA^{8,9}, viruses¹⁰, ribosomes^{11,12} and triple-stranded nucleic acid structures¹³. Polyamines also stimulate *in vitro* a wide range of different enzymatic reactions (see ref. 5 for review). The results described here show that stabilisation of condensed DNA in bacterial chromosomes by spermidine cannot be replaced even by very high concentrations of monovalent salt. It seems possible that the bifunctional character of spermidine could permit a linking interaction with two closely packed regions of a DNA double helix or at the site of an RNA-DNA association.

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IRWIN FLINK
DAVID E. PETTIJOHN

Department of Biophysics and Genetics,
University of Colorado Medical Center,
Denver, Colorado 80220

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Position of regularly spaced single-stranded regions relative to 5-bromodeoxyuridine-sensitive sites in sea urchin morula DNA

5-BROMODEOXYURIDINE (BUdR) inhibits cell differentiation and embryogenesis and yet has little effect on cell division or metabolism¹. When sea urchin embryos are grown in the presence of BUdR, pre-gastrula DNA accumulates as 30–60S duplex pieces². We have now found that naturally occurring, regularly spaced, single-stranded regions in morula DNA³ are the sites at which BUdR-induced breakage occurs, resulting in production of these 30–60S low molecular weight DNA pieces. The DNA pieces from morulae grown in BUdR are approximately half the length of DNA molecules from control embryos grown in thymidine (TdR).

In the labelled DNA preparations used at least 85% of the isolated radioactivity is covalently bonded, as determined by enzymatic and alkaline hydrolysis². Also, ³²P-DNA should represent only nuclear sequences since mitochondrial DNA is not synthesised until after the morula stage^{4,5}. Both of these points have been confirmed by CsCl buoyant density centrifugation of morula ³²P-DNA. Based on density centrifugation, we calculated that approximately 41% of the TdR residues in ³²P-DNA isolated from morulae grown in the presence of BUdR (50 µg ml⁻¹) are BUdR-substituted (S.T.C., unpublished data).

The effect of *Aspergillus oryzae* single-strand specific S₁ nuclease⁶ on the sedimentation profile of morula DNA was examined on neutral sucrose gradients. DNA was isolated from control culture embryos grown from fertilisation in the presence of 50 µg ml⁻¹ TdR and ³²P. The DNA was mixed with a reaction buffer optimised for S₁ nuclease activity and divided into two equal portions. S₁ nuclease was added to one half while the other was a control without enzyme. Both were centrifuged through sucrose gradients as described in Fig. 1. The bulk of labelled morula DNA sedimented as a single zone (Fig. 1a) at approximately 51.3S (Table 1). When previously treated with S₁ nuclease, this DNA sedimented more slowly with a peak value of 39.8S (Fig. 1b, Table 1).

The effect of S₁ nuclease treatment on the sedimentation profile of morula ³²P-DNA, isolated from embryos grown in the presence of BUdR, was also examined on neutral sucrose gradients. The major portion of this DNA sediments at about 42.7S (Fig. 2a, Table 1). After S₁ nuclease treatment, this BUdR-substituted DNA is reduced to a predominant size-class having a peak sedimentation value of 31.1S (Fig. 2b, Table 1). Thus, the bulk of the BUdR-substituted duplex DNA sedimented more slowly than the corresponding DNA

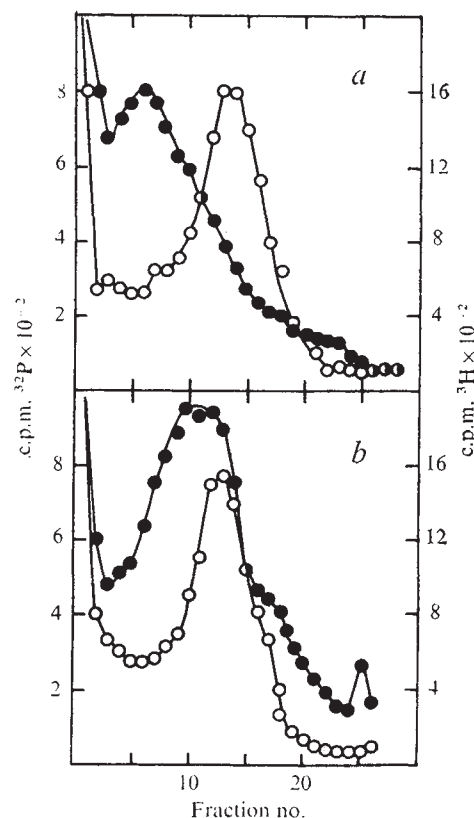


Fig. 1 Effect of S₁ nuclease on neutral sucrose sedimentation profiles of morula ³²P-DNA isolated from embryos grown in the presence of TdR: (a) ³²P-DNA incubated without addition of S₁ nuclease; (b) ³²P-DNA incubated with S₁ nuclease. *Strongylocentrotus purpuratus* embryos were cultured¹⁴ in artificial seawater¹⁵. TdR was added to 50 µg ml⁻¹ and ³²P was added to 0.5 µCi ml⁻¹ at 5 and 10 min, respectively, after fertilisation and were both present throughout the growth period. Development was terminated and DNA extracted as previously described². 200 µg of ³²P-DNA (specific activity, 650 c.p.m. µg⁻¹) was mixed with a buffer providing final concentrations of 0.15 M NaCl, 0.03 M sodium acetate, pH 4.5, and 10⁻⁴ M ZnSO₄. 25 U (ref. 16) of S₁ nuclease were mixed with 100 µg of ³²P-DNA while the remaining 100 µg of DNA was the control without S₁ nuclease. Both samples were incubated at 40° C for 45 min and the enzymatic reaction was terminated by chilling the samples and adding 10⁻¹ volume of 0.5 M EDTA. 50 µg of ³²P-DNA from each reaction mixture was combined with 10 µg ³H-TdR-labelled φ80 phage DNA, brought to a final volume of 1 ml with 0.15 M NaCl, 0.015 M sodium citrate and layered on to a 30 ml 5–20% linear sucrose gradient made in 1 M NaCl, 0.02 M Tris-HCl, pH 7.3, and 1 mM EDTA. Centrifugation was in a Beckman SW 25.1 rotor at 24,000 r.p.m. for 7 h at 20° C. Approximately 1.2 ml fractions were collected from the bottom of the centrifuge tubes and the DNA precipitated by the addition of 2 ml of 10% trichloroacetic acid and 50 µg bovine serum albumin. Samples were collected on Whatman GF/A filters and counted in a toluene-based scintillation fluid. ○, ³H; ●, ³²P.

Table 1 Effect of S₁ nuclease treatment on sucrose gradient sedimentation characteristics of ³²P-DNAs isolated from sea urchin morulae grown in the presence of either TdR or BUdR

	S ₁ treated	Neutral gradients			Alkaline gradients		
		Peak S _{20,w}	Molecular weight × 10 ⁻⁶	Base pairs × 10 ⁻³	Peak S _{20,w}	Molecular weight × 10 ⁻⁶	Bases × 10 ⁻³
TdR	—	51.3	97.7	148.1	38.9	14.8	44.8
	+	39.8	46.3	70.2	34.8	11.1	33.8
BUdR	—	42.7	57.5	87.1	38.3	14.2	43.0
	+	31.1	23.1	35.0	35.4	11.6	35.2

Neutral sedimentation data were obtained from Figs 1 and 2. Alkaline sedimentation data were obtained by centrifugation of 50 µg of sea urchin ³²P-DNA mixed with 10 µg of ³H-φ80 phage DNA. The DNAs were made 0.2 M NaOH and held at 37° C for 30 min before layering on to 30 ml 5–20% linear sucrose gradients made in 0.1 M NaOH, 0.9 M NaCl, 1 mM EDTA. Conditions of centrifugation were the same as described in Fig. 1. Conditions for S₁ nuclease treatment are given in Fig. 1. S_{20,w} values were determined by the relative sedimentation distance of ³H-φ80 phage DNA as marker, cosedimented in each gradient. Molecular weight was calculated from S_{20,w} values using the equations of Studier¹². The calculated S_{20,w} value for φ80 phage DNA is 33.4 based on a molecular weight of 28.6 × 10⁶ (ref. 13). For neutral sucrose gradients, calculations were based on continuous (gap-free) double-stranded pieces.

isolated from embryos grown in the presence of TdR. In addition, S₁ nuclease treatment reduced the sedimentation rate of the BUdR-substituted DNA even more than the enzyme affected DNA isolated from control embryos.

The effect of S₁ nuclease on sedimentation profiles obtained for morula ³²P-DNA isolated from cultures grown in the presence of TdR or BUdR was examined on alkaline sucrose gradients (Table 1). Non-enzyme-treated DNA, isolated from a control culture, sedimented as a single region of radioactivity at 38.9S. When treated with S₁ nuclease before denaturation, this DNA sedimented as a zone at 34.8S in alkali. ³²P-labelled BUdR-containing DNA had an approximate sedimentation value of 38.3S in alkaline sucrose. An equivalent sample of this latter DNA, treated with S₁ nuclease before denaturation, sediments as a sharp zone at 35.4S. While the bulk of native BUdR-substituted DNA was approximately half the length of

native DNA isolated from control embryos, alkaline denaturation of either of these DNAs resulted in molecules of approximately equal size and about one-fourth the length of the native DNA from control embryos (Table 1). Digestion of single-stranded regions by S_1 nuclease before denaturation slightly reduced the size of both the resulting single-stranded control and BUdR-substituted DNAs to approximately equal-length pieces (Table 1).

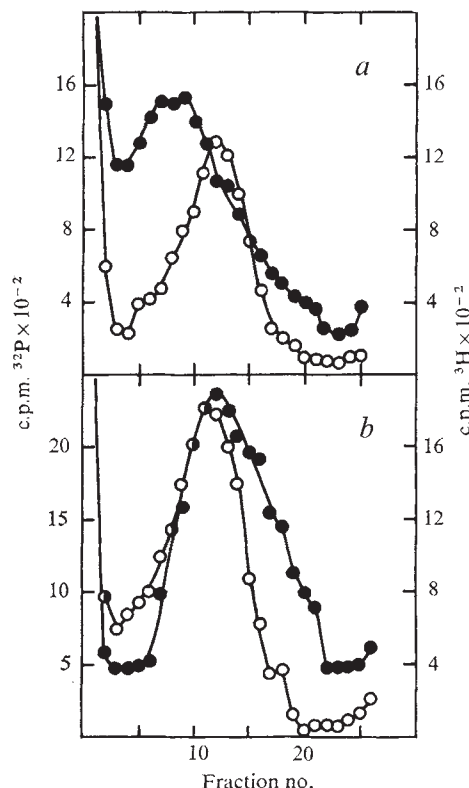


Fig. 2 The effect of S_1 nuclease on neutral sucrose sedimentation profiles of morula ^{32}P -DNA isolated from embryos grown in the presence of BUdR: (a) ^{32}P -DNA incubated without the addition of S_1 nuclease; (b) ^{32}P -DNA incubated with S_1 nuclease. Embryos were grown and ^{32}P -DNA extracted as described in Fig. 1 except that TdR was replaced by BUdR ($50\ \mu\text{g}\ \text{ml}^{-1}$). $200\ \mu\text{g}$ of ^{32}P -DNA (specific activity, $450\ \text{c.p.m.}\ \mu\text{g}^{-1}$) was mixed with NaCl-sodium acetate- ZnSO_4 ; $100\ \mu\text{g}$ of the DNA was treated with S_1 nuclease while the remaining $100\ \mu\text{g}$ was incubated without enzyme. $50\ \mu\text{g}$ of both the control and S_1 nuclease treated ^{32}P -DNAs were mixed separately with $10\ \mu\text{g}$ of ^3H -TdR-labelled $\phi 80$ phage DNA. Conditions for sedimentation and collection of samples were identical to those described in Fig. 1. $\circ$, ^3H ; $\bullet$, ^{32}P .

A model is presented in Fig. 3 for the structure of morula DNA based on the neutral and alkaline sucrose sedimentation data summarised in Table 1. While length determinations of DNA molecules on sucrose gradients are rather imprecise, the proportional molecular lengths corresponding to the peak sedimentation values obtained are in good agreement with the model. Duplex DNA molecules isolated from morula stage embryos are smaller than one would predict for chromosome-length DNA^{7,8} and thus presumably represent subunits. Each subunit of DNA from control (TdR) embryos, represented by L in Fig. 3a, must contain a centrally located S_1 nuclease-sensitive site since treatment with the enzyme produces duplex fragments approximately half the length ($1/2\text{L}$ in Fig. 3a) of the L molecule. Alkaline denaturation of the original duplex molecule (L) or the duplex fragments generated by S_1 nuclease ($1/2\text{L}$) yields single stranded DNA pieces one-fourth the length ($1/4\text{L}$ in Fig. 3a) of the original subunit. Thus, while each L molecule contains one centrally located S_1 nuclease site, each $1/2\text{L}$ molecule contains one centrally located alkali-sensitive site. The S_1 nuclease and alkali-labile sites in native DNA must

each consist of an interruption in one strand, followed closely by an interruption in the other strand of the duplex molecule. Thus, native DNA is held together at an S_1 site or an alkali-sensitive site by hydrogen bonding of the resulting overlapping cohesive single-stranded regions. If the one S_1 site and the two alkali-sensitive sites on an L molecule were the result of discontinuities in only one strand, denaturation would produce one L and four $1/4\text{L}$ single-stranded molecules. A single peak of sedimentation under alkaline conditions supports the assertion that there are discontinuities in both strands of the native L molecule. Perhaps the difference between an S_1 nuclease site and an alkali-labile site in morula DNA is that the S_1 site is a pair of adjacent gaps while the alkali-labile site is a pair of adjacent nicks. Gaps are sensitive to both S_1 nuclease and denaturation while nicks are insensitive to the enzymatic treatment under the conditions used.

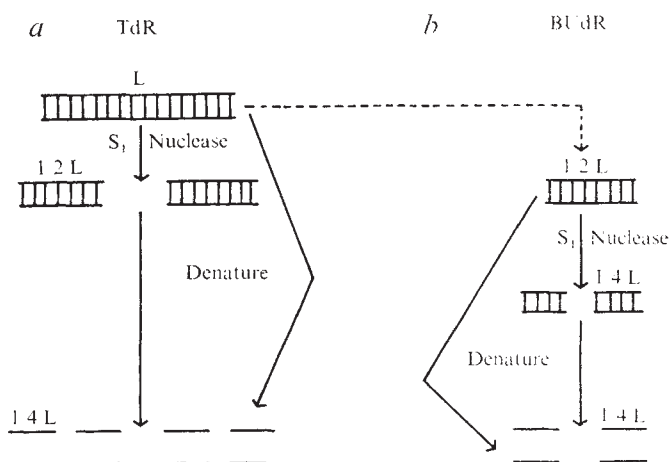


Fig. 3 A model of the relationship of regularly spaced S_1 nuclease sites and BUdR-sensitive sites in sea urchin morula DNA.

In previous work³, the average size of morula ^3H -DNA was reported to be somewhat less than that of the equivalent ^{32}P -DNA measured here. Since ^3H -radiation damage to DNA^{9,16} occurs at a faster rate than ^{32}P -radiation damage¹¹ and since the ^{32}P -DNA preparations were used as soon as possible after labelling, we believe that the larger sedimentation values reported here are more accurate.

The duplex DNA molecules isolated from embryos exposed to BUdR are equivalent in length to $1/2\text{L}$ molecules (Fig. 3b). Unlike the TdR- $1/2\text{L}$ molecules, however, the BUdR- $1/2\text{L}$ molecules have a central S_1 nuclease sensitive site. Production of BUdR- $1/2\text{L}$ molecules may have resulted from excision *in vivo* of short regions in the L molecules or possibly from a deficiency in joining of $1/2\text{L}$ molecules.

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STEVEN T. CASE
ROBERT F. BAKER

Department of Biological Sciences,
University of Southern California,
Los Angeles, California 90007

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Deficiency of the Y base in a hepatoma phenylalanine tRNA

THE structures of various tRNAs are of considerable interest as this type of RNA plays a central role in translation of the genetic code and has been shown in bacteria to function in cell regulation^{1,2}. In the search for a possible role of tRNA in regulatory processes in higher organisms, a number of studies have compared chromatographic profiles of tRNA from different tissues, organs, tumours and cell cultures³. These studies have revealed that tumour cells frequently contain tRNA species which differ in their chromatographic mobility from the corresponding tRNAs present in normal adult tissues. Until now, however, no structural basis for these tRNA differences between normal and malignant tissues has been elucidated.

One of the most consistent differences reported for tumour tRNAs involves phenylalanine tRNA (tRNA^{Phe}). Several laboratories have demonstrated by reverse phase column chromatography that certain chemically induced rat hepatomas contain, in addition to the single species of tRNA^{Phe} found in normal rat liver, an early eluting peak not normally found in rat liver^{4,5}. It is known that tRNA^{Phe} of normal eukaryotic cells contains a highly fluorescent and hydrophobic base, called Y, which is located next to the 3'-end of the anticodon⁶. The Y compound represents the most highly modified nucleic acid base characterised so far, consisting of a tricyclic imidazo derivative of guanine to which is usually attached a 4-carbon side chain⁷. In this report we present evidence that the additional species of tRNA^{Phe} found in rat hepatomas differs from normal liver tRNA^{Phe} by the absence of the Y base. Our results constitute the first elucidation of a structural difference between a single species of tRNA from malignant cells compared with the corresponding tRNA from normal cells.

Normal rat liver tRNA was aminoacylated with ¹⁴C-phenylalanine and cochromatographed on a RPC-5 column⁸ with tRNA from Morris hepatoma 7777 aminoacylated with ³H-phenylalanine. Figure 1a shows, in agreement with other

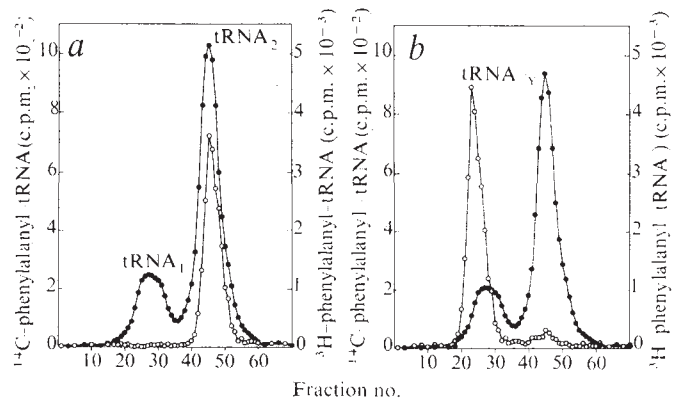


Fig. 1 Elution profiles of liver and hepatoma tRNA^{Phe} from RPC-5 columns. *a*, ³H-phenylalanyl-tRNA from Morris hepatoma 7777 cochromatographed with ¹⁴C-phenylalanyl-tRNA from normal rat liver (○). tRNA was prepared essentially according to Fink *et al.*¹⁴. Crude aminoacyl-tRNA synthetase preparations were obtained from homogenates of normal liver in medium containing 0.01 M Tris-HCl (pH 7.5); 0.1 M KCl; 5 mM MgCl₂; 0.3 M sucrose and 0.014 M β-mercaptoethanol. After removal of cellular membranes and ribosomes by centrifugation at 105,000g for 2 h, the supernatant was passed over a Sephadex G-50 column, and the void volume containing the enzymes was collected and stored in 50% glycerol at -20°C. Labelled tRNA^{Phe} was prepared by incubating 0.5-1 mg tRNA from liver or Morris hepatoma 7777 with aminoacyl-tRNA synthetase (3.1 mg protein) in a 1 ml reaction mixture containing: 10 mM KCl; 10 mM MgCl₂; 4 mM ATP (neutralised); 0.6 mM CTP (neutralised); 0.1 M Tris-HCl (pH 7.3); 0.2 mM each of nineteen amino acids (minus phenylalanine); and either 5 μCi ¹⁴C-phenylalanine (specific activity 460 Ci mol⁻¹), or 100 μCi of ³H-phenylalanine (specific activity 7 Ci mmol⁻¹) for 20 min at 37°C. The labelled tRNA^{Phe} was isolated by applying the entire reaction mixture to a 2.2 × 2.2 cm DEAE-cellulose column (DE 52 Whatman). The column was washed with 60 ml of starting buffer (50 mM acetate (pH 4.5); 1 mM MgCl₂; 0.4 mM EDTA), proteins were eluted with 20 ml 0.3 M NaCl in starting buffer, and finally tRNA^{Phe} was eluted with 10 ml 0.6 M NaCl in starting buffer. After precipitation of tRNA^{Phe} with two volumes of ethanol at -20°C, it was dissolved in 0.5 ml of starting buffer and applied to a RPC-5 column⁸. The column (0.7 × 17 cm) was eluted with a 100 ml linear gradient of 0.6-0.7 M NaCl in starting buffer at 250 pound inch⁻². Fractions (1.2 ml) were collected at a flow rate of 2 ml min⁻¹, 0.1 ml aliquots were precipitated with 1 ml 10% trichloroacetic acid at 4°C, filtered on to Millipore filters, dried and counted in a liquid scintillation spectrometer. *b*, ¹⁴C-phenylalanyl-tRNA₁ from liver (○) cochromatographed with ³H-phenylalanyl tRNA from Morris hepatoma 7777 (●). Liver tRNA (20 A₂₆₀ units) was incubated in 0.5 ml of 0.05 M citrate buffer, (pH 2.9) for 18 h at 37°C, reprecipitated with 2 ml ethanol at -20°C, aminoacylated and processed as described in (*a*).

results^{4,5}, that rat liver contained only one and hepatoma tRNA two, tRNA^{Phe} species. The major hepatoma peak (tRNA₂^{Phe}) eluted in the same position as rat liver tRNA^{Phe}.

Table 1 Poly (U)-directed ribosomal binding of hepatoma ³H-phenylalanyl-tRNAs

tRNA species	³ H-phenylalanyl-tRNA (pmol added)	pmol bound		Δpmol bound
		(-Poly(U))	(+Poly(U))	
Experiment 1				
tRNA ₁ ^{Phe}	11.7	1.9	5.9	4.0
tRNA ₂ ^{Phe}	16.6	3.1	15.4	12.3
Experiment 2				
tRNA ₁ ^{Phe}	7.8	1.4	4.4	3.0
tRNA _{2a} ^{Phe}	7.8	2.7	2.5	-0.2
tRNA _{2b} ^{Phe}	10.4	1.3	8.2	6.9

The ribosomal binding assay was essentially that of Nirenberg and Leder¹³. In experiment 1 the incubation mixture contained in 50 μl: 1.3 A₂₆₀ *Escherichia coli* ribosomes, 0.02 M Mg²⁺, 0.1 M Tris-HCl (pH 7.2), 0.05 M KCl, 0.5 μg Poly(U) and ³H-phenylalanyl-tRNA from Morris hepatoma 7777 as indicated. Incubations were carried out at 24°C for 20 min, then diluted with three volumes of the incubation buffer, filtered on to Millipore membrane filters and washed extensively with the same buffer. Radioactivity was measured in a Nuclear Chicago Mark II liquid scintillation spectrometer (1 pmol = 385 c.p.m.).

In experiment 2 the incubation system contained in 100 μl: 2.6 A₂₆₀ ribosomes, 1 μg poly(U) and hepatoma ³H-phenylalanyl-tRNAs after acid treatment and separation on RPC-5 columns as shown in Fig 2a and b. Other details were as in experiment 1.

The minor hepatoma peak (tRNA₁^{Phe}) eluted at a lower salt concentration.

The earlier elution of tRNA₁^{Phe} suggested that it might lack the very hydrophobic Y base which is present in liver tRNA^{Phe} and hepatoma tRNA₂^{Phe}. This base can be selectively excised from tRNA by mild acid treatment without breaking the polynucleotide chain⁹. tRNA modified in this way (tRNA_{-Y}) can be aminoacylated with phenylalanine, although its codon recognition is impaired^{9,10}. Figure 1b shows the results obtained when rat liver tRNA was incubated at pH 2.9 for 18 h to remove its Y base, aminoacylated with ¹⁴C-phenylalanine and cochromatographed on RPC-5 with hepatoma ³H-phenylalanyl-tRNA (Fig. 1b). After acid treatment, the normal tRNA_{-Y} eluted at a lower NaCl concentration and in a position similar to, but slightly earlier than, hepatoma tRNA₁^{Phe}.

We also isolated hepatoma tRNA₁^{Phe} and tRNA₂^{Phe} on an RPC-5 column. Each was incubated at pH 2.9 for 2 h and rechromatographed separately on RPC-5 columns. The elution position of tRNA₁^{Phe} was unchanged by the acid treatment since the peak appeared at fraction 25 before or after pH 2.9 treatment (compare Figs 1a and 2a). On the other hand, acid treatment of hepatoma tRNA₂^{Phe} (Fig. 2b) resulted in the formation of an earlier eluting fraction (tRNA_{2a}^{Phe}) corresponding to the elution position of normal liver tRNA_{-Y} (Fig. 1b). Acid treatment for 2 rather than 18 h does not completely remove the Y base (compare Figs 1b and 2b). Acid treatment of hepatoma tRNA for 18 h, however, also failed to alter the elution position of hepatoma tRNA₁^{Phe} although it converted 100% of hepatoma tRNA₂^{Phe} to earlier eluting material (separate studies not shown here). These results indicate that hepatoma tRNA₂^{Phe} contains the Y base, but hepatoma tRNA₁^{Phe} does not.

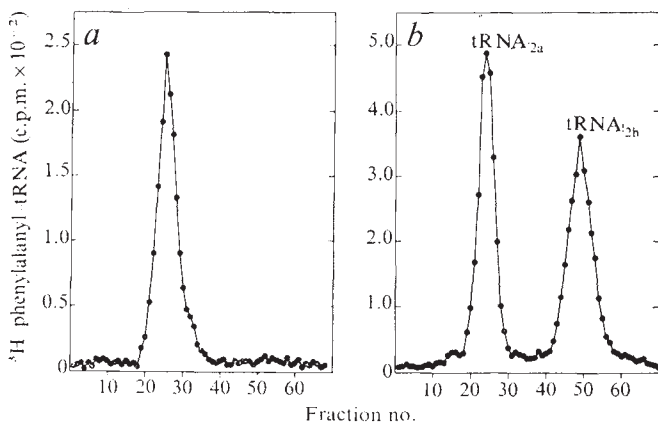


Fig. 2 RPC-5 elution profile of acid treated (a) ³H-phenylalanyl-tRNA₁ and (b) ³H-phenylalanyl-tRNA₂ from Morris hepatoma 7777. ³H-phenylalanyl-tRNA or ³H-phenylalanyl-tRNA₂ separated on a RPC-5 column (not shown here but identical to separation as in Fig. 1a) were incubated in 0.05 M citrate buffer (pH 2.9) for 2 h, directly applied to an RPC-5 column and processed as described in the legend to Fig. 1.

Since liver tRNA_{-Y} can accept phenylalanine but is impaired in ribosomal binding^{9,10} we studied poly(U)-directed ribosomal binding of the two species of hepatoma tRNA^{Phe} before and after acid treatment. In agreement with previous studies⁴ we found that before the acid treatment both species were active in this reaction (Table 1, experiment 1). When hepatoma tRNA₂^{Phe} was treated with acid, a portion of the material now eluted in the early region of the RPC-5 column (designated tRNA_{2a}^{Phe}). When this material was assayed, it failed to participate in poly(U)-directed ribosomal binding (Table 1, experiment 2). Similar results were

obtained with normal liver tRNA^{Phe} (data not shown). These findings extend previous studies with yeast tRNA^{Phe} indicating that removal of the Y base leads to loss of codon recognition^{9,10}. On the other hand, we found that acid treatment of hepatoma tRNA₁^{Phe} did not impair its ability to participate in codon recognition (Table 1, experiment 2).

These experiments indicate that hepatoma tRNA₁^{Phe} does not contain the fluorescent and hydrophobic Y base normally present in rat liver tRNA^{Phe}, as its elution position on an RPC-5 column did not change after acid treatment and it retained its binding capacity to ribosomes in the presence of poly(U).

Because excision of the base from normal liver tRNA^{Phe}, and from the major tRNA^{Phe} present in hepatomas, inactivates codon recognition we can conclude that hepatoma tRNA₁^{Phe} is not simply derived from the normal tRNA^{Phe} species as an artefact due to partial excision of the Y base during isolation of hepatoma tRNA. tRNA₁^{Phe} can function in codon recognition because, although it lacks the Y base, it presumably has another base in the position adjacent to the 3' side of its anticodon. From our present data it is not possible to decide whether the tRNA₁^{Phe} species represents the expression of a separate tRNA gene (perhaps a foetal tRNA gene¹⁵) in certain hepatomas or only an immature form of tRNA₂^{Phe} resulting from a failure to complete the synthesis of Y base from its precursor guanosine molecule^{11,12}. Nucleotide sequence studies should resolve this question and these are in progress.

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DEZIDER GRUNBERGER
I. BERNARD WEINSTEIN

Institute of Cancer Research,
and Departments of Biochemistry and Medicine,
College of Physicians and Surgeons,
Columbia University,
New York, New York 10032

J. FREDERIC MUSHINSKI

Laboratory of Cell Biology,
National Cancer Institute,
Bethesda, Maryland 20014

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Primary structure and sidechain interactions of PFL filamentous bacterial virus coat protein

Two structural classes of filamentous bacterial viruses have been identified on the basis of their X-ray diffraction patterns. The class I structure¹ is found for the fd, If1 and IKe strains, while the class II structure² is found for the Pf1 and Xf strains. The two classes differ in the number of protein subunits per turn in the virus helix (4.5 units per turn for class I and 4.4 for class II, with ~ 15 Å pitch), and in the fact that a periodic perturbation of the structure is observed for class I but not for class II. The major coat protein, which comprises about 99% of the virus coat, is largely α -helical^{3,4} with a molecular weight of about 5,000 for all strains investigated². The sequence of the fd coat protein^{4,5} is known. It is 50 residues long, with an acidic N-terminal region, a hydrophobic middle region and a basic C-terminal region. To facilitate the detailed analysis of the class II structure, and to investigate the possibility that the structural differences between class I and class II arise from differences in the coat protein, we have determined the sequence of Pf1 coat protein. This is the first system for which a molecular model of a structural protein has been described in sufficient detail to permit study of the bonding specificity between proteins. Since the α helix is a universal structure, study of the interactions between α helices can be of central importance in many unrelated systems. We have found that α helices are arranged in the virion so that hydrophobic sidechains on each protein subunit can fit into the space between sidechains on neighbouring subunits.

Pf1 virus was grown and purified as described previously², and the coat protein was purified from the virion by phenol extraction and methanol precipitation⁴.

The coat protein of Pf1 consists of 46 amino acid residues. Its amino acid composition is somewhat unusual in that it lacks proline, cysteine, histidine and tryptophan. The sequence was determined by several methods. The first 39 residues from the N terminus were established by automatic sequencing using the JOEL-JAS 47K sequence analyser. The C-terminal sequence was shown to be -Arg-Lys-Ala by carboxypeptidase A and B digestion. Treatment of Pf1 coat protein with cyanogen bromide gave three fragments containing 23, 19 and 4 residues which were separated by gel filtration and paper electrophoresis. Amino acid analysis showed that these fragments accounted for the entire coat protein. It also facilitated the assignment of the 19-residue fragment as the N terminus and the 4-residue fragment as the C terminus. The structure of the 23-residue fragment was determined by automatic sequencing. These results were confirmed by the isolation and sequencing of the five chymotryptic peptides obtained from a digest of the succinylated protein. The results of these determinations showing the location of the cyanogen bromide fragments and chymotryptic peptides, and sequences established by Edman degradation and carboxypeptidase digestion, are shown in Fig. 1.

The coat protein sequence of Pf1 is compared with that of fd in Fig. 2. The sequences are aligned on the basis of similarities in the predicted secondary structure. With this alignment, the average minimum base change per codon⁶ required to go from the Pf1 to the fd sequence would be 1.24. Although fd and Pf1 might be related by convergent evolution, this seems unlikely in view of the complexity of the virions. If the two viruses are related by divergent evolution, a time scale for evolution⁷ calibrated on minimum base change per codon suggests that the two viruses would share a common ancestor in existence about 3×10^9 yr ago. The similarity² between the A proteins

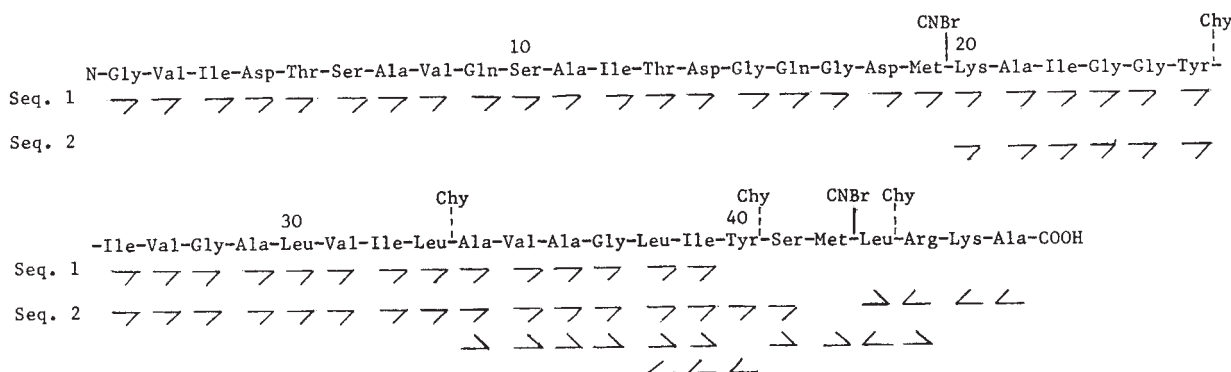


Fig. 1 Determination of the Pf1 coat protein sequence. Seq. 1 and seq. 2 ($\rightarrow$) are determinations using the JOEL-JAS 47K sequence analyser; ($\rightarrow$) are sequences determined by dansyl-Edman degradation, and ($\leftarrow$) are sequences established by carboxypeptidase A and B digestion. CNBr and Chy indicate sites of cleavage by cyanogen bromide and chymotrypsin, respectively.

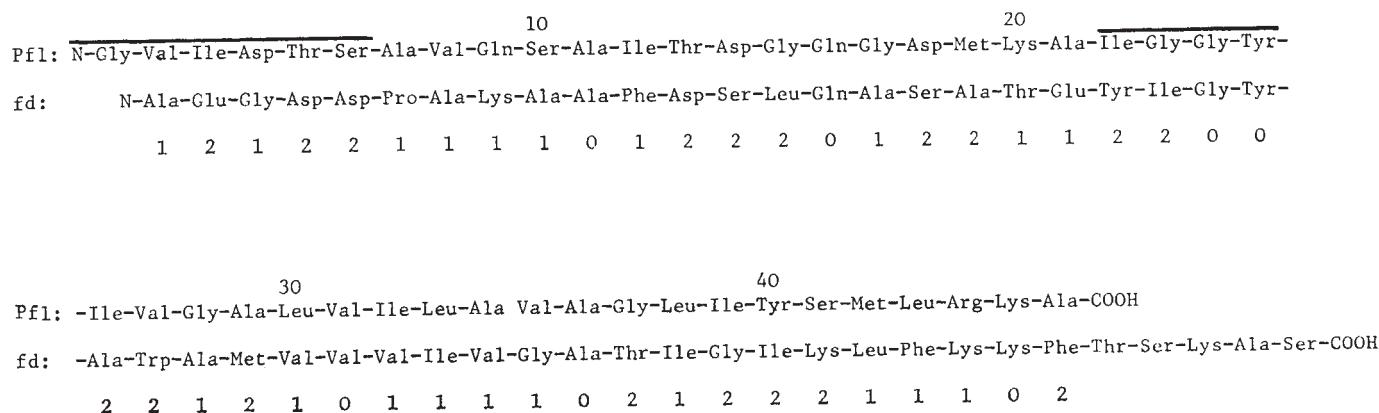


Fig. 2 Comparison of the Pf1 and fd coat protein sequences. Sequences were aligned on the basis of the positions of non-helical residues and the positions of basic residues at the C terminus. The regions identified by bars have low probability of α helix⁶. The numbers in the third row indicate the minimum base change per codon required to give the observed amino acid change, taken from Table 2 of ref. 6.

Spectroscopic measurements on fd^{3,4} and Pf1 (R.L.W. and

L. A. Day, in preparation) show that more than 90% of the residues in the coat protein are in the α -helix conformation. To identify possible non-helical regions, the observed probability of finding residues in α -helical regions (P_{α}) or β sheet regions (P_{β}) was used to predict the secondary structure from the sequence⁹. The only two regions of four or more residues with average P_{α} less than one are indicated by bars in Fig. 2. The Pro-6 of fd is included at the N terminus of the α -helical region as observed for several structures⁹. Residues 22–25 of

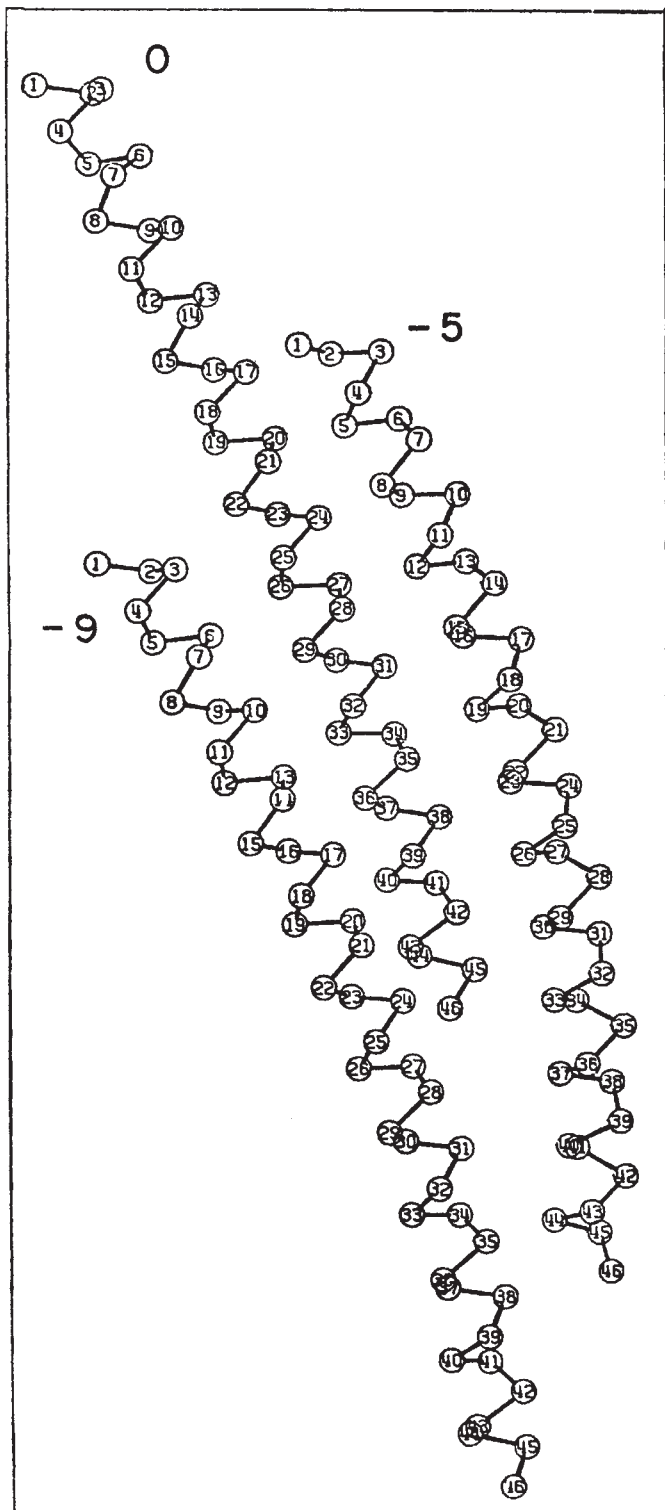


Fig. 3 Ball-and-stick representation of α carbon atoms in neighbouring α helices of the current Pfl model. Atoms are projected on to a plane parallel to the z axis of the virion, to give a view similar to that shown in Fig. 5 of ref. 10. Numbers indicate residues. The three subunits illustrate the contacts between any subunit (0) and the subunits five units (-5) and nine units (-9) down the virus helix.

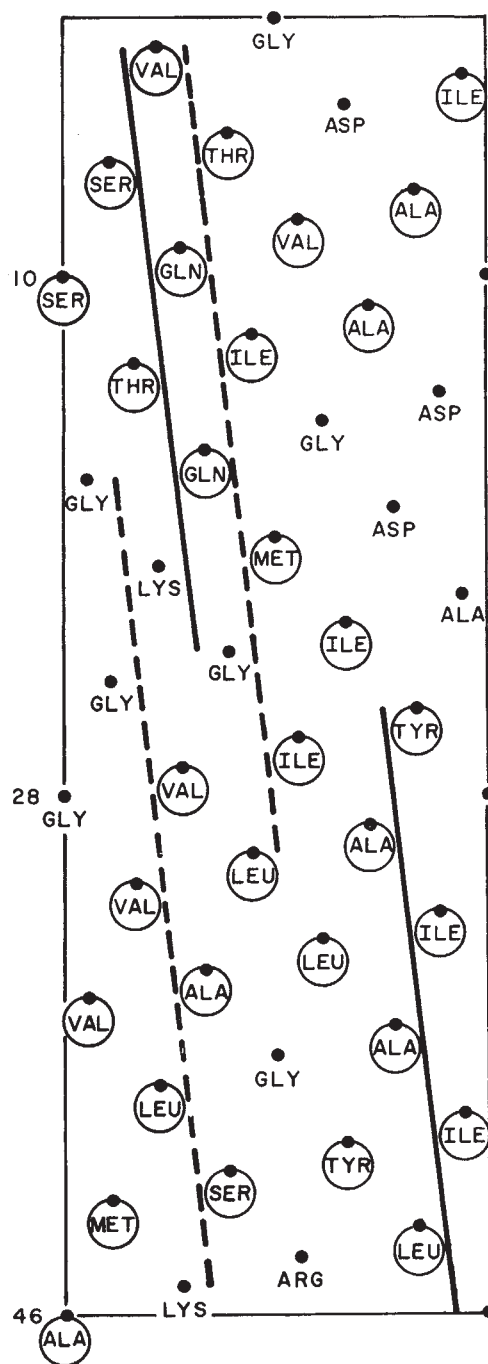


Fig. 4 Surface lattice of the Pf I α -helical coat protein. Positions of residues are projected on to a cylinder which is opened flat and viewed from the outside. Hydrophobic residues or residues involved in van der Waals' contacts are circled (the classification of glutamine, serine and tyrosine as hydrophobic is discussed in ref. 14). Dashed lines indicate the lines of contact between the 0 and -5 molecules, and solid lines indicate the lines of contact between the 0 and -9 molecules. In each case the lower line represents the line of contact on the 0 molecule, and the upper line represents the contacts on the other molecule (see also Fig. 3).

Table 1 Side-chain interactions in the Pf1 model

Residues	Helices 0 to -5 Position*	Type†	Distance (Å)†	Residues	Helices 0 to -9 Position*	Type†	Distance (Å)†
17-2	I	—	6.8G	25-2	II	●	>7.0
20-5	II	—	6.4	29-6	I	—	4.5
24-9	I	—	6.0G	32-9	II	●	>7.0
27-12	II	●	5.9	36-13	I	●	4.3
31-16	I	●	5.1	39-16	II	●	6.4
34-19	II	●	5.7	43-20	I	○	4.4
38-23	I	—	4.6G	46-23	II	—	4.8G
41-26	II	●	5.7				
45-30	I	○	4.4				

* Position I and position II refer to the different kinds of sidechain contact on the left and right of the line of contact, as defined in ref. 14. A residue in position I is 3 residues on the N-terminal side and 4 residues on the C-terminal side of a residue in position II.

† ●, Van der Waals' contact between β - and γ - or δ -carbons and hydrogens, not involving charged residues. ○, Van der Waals' contact between β - and γ - or δ -carbons and hydrogens, involving charged residues. —, no β - to γ - or δ -carbon and hydrogen van der Waals' contact. Taken from Figs 8 and 9 of ref. 14.

† Distance between β -carbons. Where one of the residues involved in the contact is glycine (indicated by G), the distance is given to the position that would be occupied by β -carbon if the residue were alanine.

Pf1 (21-24 of fd) could represent a bend in an α helix involving breakage of a few hydrogen bonds, rather than a drastic disruption of the helix. The non-helical region at the N terminus is sufficient to account for the observed fraction of non-helical residues. The N terminus is probably at the outside surface of the virion¹⁰. This non-helical region is reminiscent of the globular head group at the N terminus¹¹ of the α -helical myosin molecule, situated at the outside surface of myosin filaments in muscle.

For study of sidechain interactions, the Pf1 coat protein can be represented as a 46-residue rod of α helix. The placement of the coat protein rods in the virion¹⁰ shows that there are two main types of interaction between proteins: from each protein to the proteins originating 5 and 9 units down the ~ 15 Å virus helix (Fig. 3). The axes of neighbouring α helices make an angle of 9-12° with each other over most of the 40 Å interaction region¹⁰. Analysis of knobs-into-holes interaction of sidechains on infinitely long idealised α helices shows that the sidechains of one helix can fit into the space between sidechains on its neighbour only if the two helices wind around one another with an angle of 20° between the helix axes¹². The region of interaction between Pf1 coat proteins, however, is short enough to permit knobs-into-holes packing over the whole of the interaction region in spite of the small crossing angle.

The remarkable resistance¹³ of filamentous bacterial viruses to extremes of pH suggests that the α helices are held together by hydrophobic bonds rather than salt bridges or hydrogen bonds. The details of sidechain interactions were therefore examined using the principles of van der Waals' interaction developed for tropomyosin coiled-coils¹⁴. Sidechain interactions were explored for several rotations of the α -helix subunit about its own axis (all subunits were assumed to have the same orientation). One orientation was clearly better than all others tried (Fig. 4). Most hydrophobic residues (24 out of 32) are directly involved in interactions with residues on neighbouring α helices (this number includes Val-35 and Met-42, residues that are involved in the shorter interaction region between the 0 and the -14 subunits). Not only the general hydrophobicity of the residues but also the detailed stereochemistry of the interactions is acceptable with this orientation (Table 1). Ten out of the sixteen interactions are of the most favourable type¹⁴, involving van der Waals' contact between β - and γ - or δ -carbons and hydrogens; of these only two involve charged sidechains. The remaining six interactions are of a type that would not permit van der Waals' attraction for regularly aligned α helices, but three of these are in the possible non-helical regions (residues 1-6). For two more, the bending of the α helix in the Pf1 model causes β -carbons to approach to within less than 5 Å of each other, so that van der Waals' attraction might even be possible at these positions.

The coat proteins of both class II viruses, Xf¹⁵ and P1, are about 10% smaller than those of the class I viruses fd and If1 (51 residues: Y.N., unpublished). The perturbation observed in

the structure of class I but not class II virions might be due to the additional four or five residues at the C-terminal end of the coat protein. The C-terminal ends are presumed to be in the interior of the virus shell¹⁰, so that additional material here could lead to interference and thus to a structural perturbation.

The model-building approach to molecular structure requires that any acceptable model not only gives a calculated Fourier transform sufficiently close to the observed X-ray data, but also makes stereochemical sense at a level beyond the resolution of the X-ray data. The model-building approach has in the past been restricted to structures made of covalently bonded subunits, such as helical polypeptides or polynucleotides. But this approach can also be useful for the filamentous bacterial viruses, even though the subunits are not covalently linked, because of the knowledge available about internal stereochemistry of α helices and about van der Waals' interactions between α helices. The analysis of sidechain interactions presented here indicates that the model proposed for the Pf1 virion is indeed stereochemically acceptable.

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Y. NAKASHIMA
R. L. WISEMAN*
W. KONIGSBERG
D. A. MARVIN

Department of Molecular Biophysics and Biochemistry,
Yale University,
New Haven, Connecticut 06520

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* Present address: Public Health Research Institute of the City of New York, New York, New York 10016.

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Vaccination of non-human primates against malignant lymphoma

HERPESVIRUSES are the most important candidates for human cancer agents. The Epstein-Barr virus (EBV) in particular is suspected of being the causal agent in Burkitt's lymphoma and nasopharyngeal carcinoma¹. Immunological principles, well understood from vaccine prophylaxis of viral diseases, provide the potential for the eventual control of herpesvirus-induced neoplasia. The development of safe and efficient cancer vaccines with the human candidate herpesviruses is, however, difficult, as there are no reliable *in vitro* or experimental animal methods for detecting oncogenic potential in preparations which might be applicable to the human subject. We are studying the problems of herpesvirus cancer vaccines in non-human primates. Two members of the herpesvirus family, *H. saimiri* (HVS) and *H. ateles* (HVA), induce malignant lymphoma and leukemia in several New World monkey species^{2,3}. HVS-induced lymphoma in cotton topped (CT) marmoset monkeys (*Saguinus oedipus*) has been used in this study as all monkeys inoculated with HVS regularly develop malignant lymphoma within two months^{4,5}. This investigation describes the effect of a killed herpesvirus cancer vaccine in protecting non-human primates against malignant lymphoma.

in owl monkey kidney (OMK) cell cultures with a constant serum dilution (1:5) and varying tenfold virus dilutions according to standard techniques⁷. Three of the actively immunised monkeys (A, B and C) have been under observation for more than a year (581 days). The serum antibodies appeared 3-4 weeks after the first immunisation and reached their maximal titres 2-3 weeks later. The antibody titres slowly dropped during the first year and rose again after a booster injection (Table 1).

Out of the 42 immunised monkeys 22 were challenged with HVS. The stock virus, grown in OMK cells⁸, was passed through a Millipore membrane filter (450 nm) and serial tenfold dilutions of the filtrate were prepared. Aliquots (1 ml) of each dilution were injected intramuscularly into immunised monkeys and also into non-immunised control monkeys. The infectivity titre (TCID₅₀) of the virus dilutions was determined in parallel in OMK cells.

In the first experiment three immunised monkeys were challenged with the 10⁻³ dilution of the stock virus and three monkeys with the 10⁻⁴ dilution. The TCID₅₀ of the 10⁻³ dilution was 10 and corresponded to 18 LD₅₀ calculated according to the method of Reed and Muench. All of the vaccinated monkeys survived without any signs of disease and have now been under observation for 337 days. All of the non-immunised monkeys which received the 10⁻³ dilution and two out of three control monkeys which were inoculated with the 10⁻⁴ dilution died of malignant lymphoma 34-51 d after inoculation, indicated by gross pathology and histopathology (Table 2). The tumour-bearing animals carried the HVS genome in their peripheral white blood cells as shown by cocultivation with OMK cells and they developed serum antibodies against HVS. In contrast to the tumour-bearing control monkeys, the HVS could not be extracted from fresh peripheral white blood cells of the vaccinated monkeys by cocultivation methods.

Table 1 Development of serum antibodies against HVS in CT marmoset monkeys immunised with killed HVS

Animal A			Animal B			Animal C		
Days after first vaccination	Serum antibodies against HVS		Days after first vaccination	Serum antibodies against HVS		Days after first vaccination	Serum antibodies against HVS	
	NI*	CF†		NI*	CF†		NI*	CF†
14	0.0	c	13	0.0	c	18	1.0	c
27	1.5	c	26	1.7	c	26	2.3	c
40	IV 3.3	1:32	39	3.2	c	42	IV 3.5	1:64
73	IV 3.3	1:16	60	3.2	c	56	IV 3.5	1:64
97	IV 3.3	1:8	83	2.2	c	123	2.5	c
208	IV 3.3	c	153	1.5	c	152	2.2	c
409	IV 2.0	c	222	2.0	c	180	3.5	c
444‡	1.5	c	395	1.2	c	388‡	0.7	c
452	2.8	c	433‡	0.5	c	396	1.8	1:16
			441	3.0	c			

*Neutralisation indices.

†Titres of complement fixing antibodies.

c, Serum had anticomplementary activity.

‡The monkey received a booster injection of killed HVS.

The killed vaccine was prepared by inactivation of HVS with heat (56°C for 4 h) and formaldehyde (100 γ ml⁻¹ for 6 days) as previously described⁶. The HVS-specific antigenicity of the killed vaccines was determined by the complement fixation (CF) test according to standard procedures⁷. An HVS-specific antiserum from an owl monkey latently infected with HVS⁸ was used in the CF test. The CF titres of the vaccines ranged between 1:32 and 1:64. Before use, the vaccines were adsorbed on to Aluminiumhydroxygel (10% v/v of a 3% solution, Behringwerke, Marburg, West Germany) as adjuvant. For immunisation four to six intramuscular inoculations of the vaccine were given to each monkey within 4-10 weeks, starting with 1.0 ml vaccine for the first inoculation and 0.5 ml for each of the subsequent inoculations. The 42 CT marmoset monkeys actively immunised with the vaccine remained clinically well and developed high titres of neutralising and CF antibodies against HVS. The neutralisation tests were performed

In the second challenge experiment a higher dosage of HVS was used. A 10⁻² dilution of the stock virus was given to four vaccinated monkeys and another four vaccinated monkeys received a 10⁻³ dilution. The 10⁻² dilution contained 100 TCID₅₀ which corresponded to 215 LD₅₀. All of the challenged monkeys were resistant to infection with HVS (Table 2) and have now been under observation for 206 days without any indication of tumour development. All of the non-vaccinated controls which received the 10⁻³ dilution and three out of four monkeys which received the 10⁻⁴ dilution died of malignant lymphoma within 36-52 d of inoculation.

In the third experiment the 10⁻² and 10⁻³ dilutions of the stock virus were again used to challenge four vaccinated monkeys in each group. Since the first and second experiment had shown that 1 TCID₅₀ of HVS corresponded to about 2 LD₅₀ the titration of the inocula in non-vaccinated control monkeys was omitted. Both groups of monkeys in this third

Table 2 Challenge experiments 1–3: CT marmoset monkeys vaccinated with killed HVS and non-vaccinated control monkeys were inoculated intramuscularly with serial tenfold dilutions of cell-free HVS

Dilution of HVS stock virus	Non-immunised monkeys			Immunised monkeys			Titre of inoculum LD ₅₀	*TCID ₅₀
Experiment 1								
10 ⁻³	+	+	+	—	—	—	18	10
10 ⁻⁴	+	+	—	—	—	—	1.8	1
10 ⁻⁵	—	—	—	not done			≤1	≤1
Experiment 2								
10 ⁻²	not done			—	—	—	215	100
10 ⁻³	+	+	+	—	—	—	21	10
10 ⁻⁴	+	+	—	not done			2	1
10 ⁻⁵	—	—	—	not done			≤1	≤1
Experiment 3								
10 ⁻²	not done			—	—	—	90	
10 ⁻³	not done			—	—	—	9	

+, Monkey died of malignant lymphoma.

—, Monkey remained clinically well.

*, Determined in OMK cells.

experiment which were challenged with 9 or 90 TCID₅₀, corresponding to about 18 or 180 LD₅₀, have now been under observation for 165 d. The monkeys have remained clinically well and do not show any signs of tumour development (Table 2).

These experiments clearly demonstrate that a malignant tumour in non-human primates can be prevented by a vaccine. The neoplasia investigated is a malignant lymphoproliferative disease induced by inoculation of cell-free HVS into CT marmoset monkeys under laboratory conditions. Under natural conditions HVS appears to be transmitted horizontally, similar to other herpesviruses which have been associated with neoplasia. The prophylaxis of the malignancy was achieved with a killed vaccine prepared from the oncogenic HVS by inactivation. The vaccine proved to be safe and efficient as yet. All vaccinated monkeys developed high titres of humoral antibodies against HVS and remained clinically well. The immunity against HVS can be broken, however, by very high challenge dosages, such as 10^{3.8} TCID₅₀ of HVS⁸. Experiments in progress indicate that the vaccinated monkeys are not resistant to malignancy caused by tumour cell transplantation. The tumour prophylaxis with the killed HVS vaccine and the finding that the TCID₅₀ of HVS correlated very well with the LD₅₀ of HVS support the concept that HVS is indeed the aetiological agent of malignant lymphoma in monkeys. The preparation of killed vaccines may be advantageous with respect to the candidate human cancer herpesviruses since it will be very difficult to exclude oncogenicity of live attenuated vaccines before clinical trials. Recently two groups of investigators showed that EBV-induced lymphoid tumours in *S. oedipus*⁹ and in an owl monkey¹⁰. A killed EBV vaccine may be prepared and tested in non-human primates analogous to the HVS cancer vaccine.

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R. LAUFS
H. STEINKE

Hygiene-Institut der Universität,
D-34 Göttingen, Kreuzberggrüß,
West Germany

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***In vitro* absorption and molecular weight of specific T-cell suppressor factor**

THE lymph node cells of mice rendered unresponsive by the injection of picryl sulphonic acid (PSA) depress the passive transfer of contact sensitivity¹. When the mice are also painted with picryl chloride the lymph node cells produce suppressor factor *in vitro*. This factor depresses the passive transfer of contact sensitivity by immune cells and its production is T-cell dependent². Here we show that suppressor factor(s) has a molecular weight ~50,000 and that it is absorbed by and can be eluted from specific antigen in the form of picrylated albumin attached to Sepharose beads.

Suppressor factor was prepared by injecting CBA mice intravenously with 5 mg neutralised PSA (BDH). The abdomen and forepaws were painted with 0.15 ml 7% picryl chloride (PCI) in ethanol 5 d later. The draining lymph nodes were taken the next day and live cells (1.2×10^7 ml⁻¹) were incubated in Eagle's minimal essential medium (Difco) containing 10% foetal calf serum, penicillin, streptomycin and glutamine for 48 h. The gas phase was O₂ 7%; N₂ 83%; CO₂ 10%. The supernatants were collected by centrifugation (300g, 10 min) followed by top speed on a bench centrifuge for 30 min. Control (inactive) supernatants were prepared from mice which were painted with PCI but had not received PSA.

In the direct test² the fractions were tested for their ability to inhibit the passive transfer of contact sensitivity to PCI by incubating immune cells in them (Table 1). They were also tested in the indirect test² which is based on the fact that normal peritoneal exudate (PE) cells incubated in suppressor supernatant inhibit the passive transfer of contact sensitivity by immune cells. Five day oil-induced (light liquid paraffin) PE cells were incubated with fractions (1.2×10^7 ml⁻¹, 1 h, 37° C).

Table 1 Absorption and elution of suppressor factor from picrylated and 'oxazolinated' albumin on Sepharose beads

	Increment of ear thickness $\pm$ s.d. at	
	24 h	48 h
Control (inactive) supernatant	4.1 $\pm$ 0.65	5.2 $\pm$ 0.68
Suppressor supernatant	1.8 $\pm$ 0.51	2.3 $\pm$ 0.49
Suppressor factor absorbed with Pic beads*	4.0 $\pm$ 0.62	5.0 $\pm$ 0.78
Suppressor factor absorbed with Ox beads*	2.3 $\pm$ 0.59	2.8 $\pm$ 0.58
Eluate from Pic beads	2.2 $\pm$ 0.84	2.9 $\pm$ 0.72
Eluate from Ox beads	3.9 $\pm$ 0.47	4.6 $\pm$ 0.50
Control supernatant eluted from Pic beads	3.6 $\pm$ 0.56	4.8 $\pm$ 0.79
Negative control (non-specific swelling)	1.5 $\pm$ 0.54	1.6 $\pm$ 0.30

The supernatants were absorbed three times by picrylated (Pic) or oxazolinated (Ox) Sepharose beads. The supernatants and eluates were assessed in the direct test by incubation with 4 d PCI immune cells (5×10^7 ml $^{-1}$, 1 h, 37° C). They were washed twice and 4×10^7 cells were injected into each of five recipients. The mice were challenged with 1% PCI in olive oil and passive transfer assessed by the increment of ear thickness. This experiment was repeated on two additional occasions with similar results but the Ox-bead absorptions were not undertaken.

* Bovine serum albumin (1%) beads was coupled to activated sepharose 4B beads at pH 6.2 (ref. 3). Picrylation: 0.1% PSA, pH 9.5, 48 h, room temperature. Oxazolination: 10 ml beads, 20 ml 0.2 M borate buffer pH 8.4, 0.2 and then 0.3 ml, 5% 4-ethoxymethylene-2-phenyloxazolone (BDH) in ethanol, squirted from a syringe, 18 h. The beads were washed with 0.1% Tween, pH 2.3 0.2 M glycine-HCl buffer, and PBS.

They were spun down, washed twice and mixed with 4 d immune cells and injected into groups of five mice (4×10^7 immune cells; 1.2×10^7 PE cells per recipient).

In a typical experiment, mice injected with immune cells incubated in tissue culture medium ($CS_{100\%}$) give a mean increment of ear thickness of 4.4 units (10^{-3} cm), after challenge with PCI, while mice which received no cells ($CS_{0\%}$) give a mean increment of 1.4 units. The inhibition caused by a fraction (CS_{exp}) is expressed as percentage inhibition

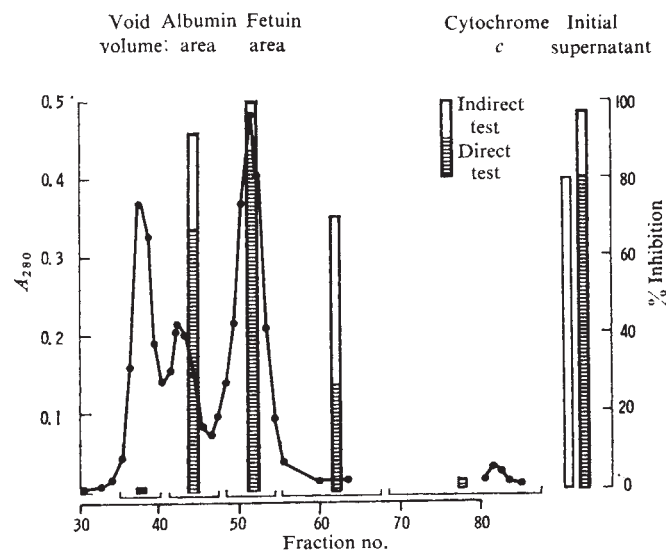
$$(CS_{100\%} - CS_{exp}) / (CS_{100\%} - CS_{0\%}) \times 100$$

Figure 1 shows a Sephadex G-100 experiment. The initial suppressor supernatant gave 97% inhibition. Most of the activity in the direct test (87% inhibition) occurred in the fetuin area (molecular weight, 48,000) with less activity in the albumin area (molecular weight 67,000). A little activity eluted after fetuin (27% inhibition). These results were confirmed in the indirect test which also showed activity eluting after the fetuin area.

Fractions from control supernatant were virtually inactive. The only inhibition found in the direct test was 7% in the

albumin area. In the indirect test, 10% inhibition occurred in the albumin area and 3% in the fetuin area.

These results were confirmed in six additional gel filtration experiments. Table 2 shows that in the direct test using fractions from a G-200 or G-100 column there was little or no activity in the void volume and the maximal activity was in the fetuin area. With G-50 an average of 77% inhibition occurred in the void volume and a mean of 90% with material which

**Fig. 1** Gel filtration of suppressor supernatant on Sephadex G-100.

eluted later. The G-50 data suggest that there may be material with a molecular weight below fetuin. This may have been missed in the G-100 experiment because of the loss of material which occurs on freeze-drying from dilute solutions.

The indirect test gave the same general pattern of results but the inhibition in the IgG area was greater. These results were confirmed using Amicon filters PM-100, XM-50 and UM-10. The fractions with presumptive molecular weight above 100,000 and below 10,000 were inactive. Material lying between 100,000 and 50,000 daltons gave 66% inhibition while material between 50,000 and 10,000 daltons gave 89% inhibition.

It was concluded that suppressor factor behaved as though it had a molecular weight close to that of fetuin (48,000). It is probably polydisperse. It is not clear whether the material with

Table 2 Suppressor activity of different gel filtration fractions in the direct and indirect test

Sephadex*	Experiment†	Initial supernatant	% Inhibition of contact sensitivity				Low molecular weight area
			IgM area	IgG area	Albumin area	Fetuin area	
G-200	1	69(88)‡	6(28)	17(32)	57(53)	80(85)	NA
	2	90	6	23	87§		
G-100	1	69		20	71	83	NA
	2	90(88)		10 (32)	42(97)	84(88)	NA
	3¶	97(82)		0	67(97)	87(100)	27(70)
G-50	1	69				77	89
	2	90(88)				65 (94)	90(100)
Average			6	14(32)	58(82)	75(92)	69(85)

*16 ml supernatant on columns of 1,600 ml, diameter 3.7 cm, eluted with 0.1 M NH_4HCO_3 , freeze-dried and reconstituted with 8 ml tissue culture medium shortly before use.

†Experiments with the same number used the same initial supernatant and were tested simultaneously.

‡The number in brackets refers to the indirect test.

§The albumin and fetuin areas were combined.

||Void volume

¶Experiments also shown in Fig. 1.

a molecular weight in the IgG and IgM area detected in the indirect test is an antibody or antibody-antigen complex or a low molecular weight T-cell product combined with a high molecular weight picrylated protein.

The following experiments provide immunochemical evidence for the specificity of suppressor factor and show that it can be specifically absorbed by and eluted from picrylated but not by 'oxazoloned' protein bound to Sepharose beads.

Suppressor supernatant (16 ml) was absorbed on to picrylated or oxazoloned beads. For elution the beads used for the first absorption (8 ml) were washed four times in 200 ml ice cold saline and resuspended in 3 ml tissue culture medium with 1% foetal calf serum and heated at 56°C for 30 min. The supernatant was removed and 3 ml of 2 M NaCl added. This was removed after 15 min at room temperature and followed by 0.1 M, pH 2.7 glycylglycine-hydrochloric acid buffer for 15 min. The eluates were dialysed against phosphate buffered saline, pH 7.2, followed by tissue culture medium. Foetal calf serum was added to 5% and the eluate assessed in the direct test.

Table 2 shows that suppressor activity was specifically absorbed by and eluted from picrylated beads. This specific absorption shows that this suppressor factor is not an anti-receptor site factor of the type described by Ramseier⁴. It is not known whether the suppressor factor is a specific T cell product or a complex of the specific T cell product and antigen. In the latter case the molecular weight of the T cell product without antigen is less than about 50,000 daltons and may be much lower.

The suppressor factor described here has a similar molecular weight to the suppressor factors which specifically prevent a tumour rejection (S. Fujimoto, M. Green, and A. H. Sehon, unpublished) and specifically depress the homocytotrophic (presumptive IgE) antibody response⁶. Purification by gel filtration and absorption and elution from antigen opens up the possibility of detailed immunochemical studies.

We wish to thank the Medical Research Council for facilitating this work and the Polish Academy of Science for financial help, and Nortin Hadler for technical advice.

M. ZEMBALA

Department of Medical Microbiology,
Institute of Microbiology,
Academy of Medicine,
31-121 Krakow,
Ul. Czysa 18,
Poland

G. L. ASHERSON

Division of Immunology,
Clinical Research Centre

BARBARA MAYHEW

Division of Immunology,
Clinical Research Centre,
Watford Road,
Harrow HA1 3UJ, Middlesex, UK

J. KREJCI

Institute of Sera and Vaccines,
W. Pieck Street 108,
Praha 10,
Czechoslovakia

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Errata

In the article "Stimulation of synaptosomal dopamine synthesis by veratridine" by R. L. Patrick and J. D. Barchas (*Nature*, **250**, 737; 1974) the following corrections are necessary. Paragraph 2, line 6 should read . . . fraction has been shown to be associated with the synaptosomal component . . . and not as printed. In line 4 of the last paragraph interact should read intact. There were also errors in the Tables which, for the sake of clarity, are reprinted in full below.

Table 1 Effect of tetrodotoxin on the veratridine-induced increase in dopamine synthesis

	Dopamine synthesis (nmol h ⁻¹ g ⁻¹)
Controls	9.54 ± 0.53 (12)
Tetrodotoxin	9.50 ± 0.57 (12)
Veratridine	14.7 ± 0.46 (12)*
Tetrodotoxin + veratridine	10.5 ± 0.43 (12)

Aliquots of the striatal P₂ fraction were incubated for 5 min at 37°C either without further additions or in the presence of tetrodotoxin (2 × 10⁻⁷ M) followed either by the simultaneous addition of veratridine (7.5 × 10⁻⁵ M) plus L-1-¹⁴C-tyrosine (1 × 10⁻⁵ M) or by the addition of tyrosine alone, and incubated for an additional 5 min. The apparent rate of synthesis was calculated by dividing the d.p.m. of product formed per hour per gram of original tissue by the specific activity of the tyrosine added to the medium. The normal incubation medium had the following composition: NaCl, 125 mM; KCl, 5 mM; CaCl₂, 1 mM; MgCl₂, 1 mM; glucose, 10 mM; ascorbic acid, 1 mM (made fresh daily); and Tris-HCl, 50 mM pH 7.4. Values represent the mean ± s.e.m. The number of observations is in parentheses.

*P < 0.001 controls, P < 0.001 tetrodotoxin plus veratridine (t test).

Table 2 Calcium-dependence of the veratridine-induced increase in dopamine synthesis

	Dopamine synthesis (nmol h ⁻¹ g ⁻¹)		
	+ Ca ²⁺	- Ca ²⁺ + EGTA	- Ca ²⁺
Controls	13.8 ± 0.22 (18)	13.8 ± 0.41 (18)	12.8 ± 0.72 (10)
Veratridine	18.1 ± 0.30 (18)*	14.4 ± 0.44 (18)	12.0 ± 0.82 (10)

Aliquots of the striatal P₂ fraction were incubated for 5 min at 37°C either in control medium, Ca²⁺-free medium containing 1 mM EGTA, or simply Ca²⁺-free medium, followed by the simultaneous addition of veratridine (7.5 × 10⁻⁵ M) plus L-1-¹⁴C-tyrosine (1 × 10⁻⁵ M) or by the addition of tyrosine alone, and incubated for an additional 5 min. Values represent the mean ± s.e.m. The number of observations is in parentheses.

*P < 0.001 controls.

In the article "Dissociation of EEG and behavioural effects of ethanol provides evidence for a noncholinergic basis of intoxication" by W. R. Klemm (*Nature*, **251**, 234; 1974) the following corrections should be made. In the legend to Fig. 1, the dose of eserine (Fig. 1b) should be 0.2 mg kg⁻¹, and of alcohol (Fig. 1c) should be 2 g kg⁻¹. Likewise on page 235, right-hand column, line 27, the dose should be 2 g kg⁻¹.

In the article "Radiocarbon chronology for Seibal, Guatemala" by R. Berger, S. de Atley, R. Protsch and G. R. Willey (*Nature*, **252**, 472; 1974) the first word in line 4, para 1 should read 'stages' and we erroneously gave the address of R. Protsch as Frankfurt am Main, DDR, when in fact it should be West Germany.

reviews

The Social Behaviour of the Bees: A Comparative Study. By Charles D. Michener. Pp. xii+404. (The Belknap Press of Harvard University: Cambridge, Massachusetts, July 1974.) \$25.00.

THE conspicuous social behaviour of bees has undoubtedly been the main reason why they have always stimulated much attention, and continue to do so, with the result that they are kept in the forefront of entomological research. It is indeed a challenging task to attempt to synthesise the accumulated research findings on social bees, and it is one which Professor Michener has ably and brilliantly fulfilled. This book is timely and appropriate and the wealth of recent knowledge gleaned from many sources and many studies is interwoven skilfully around the recurring theme of the origin and evolution of social life to produce a masterly and fascinating exposition. Readers are especially fortunate that Professor Michener's deep and wide knowledge of his subject enables him to put current or fashionable ideas into an objective perspective.

The arrangement of the subject matter of the book into three parts is unusual but effective. Part I is a useful introduction for those unfamiliar with bees. It includes background material about their development, discussion of the features that seem to be involved especially in social behaviour (for example, mouthparts, sting, pollen collecting apparatus and various glands), evolution and classification, and the terminology commonly used.

Bees are unique among social insects in exhibiting a broad spectrum of stages illustrating the evolution of social behaviour. In Part II—the largest part of the book—the types and levels of bee societies and the origins and growth of aggregations and colonies are discussed. Several topics are then taken in turn and for each topic bees exhibiting different stages of social organisation are considered. These topics include the nest and its contents, control of the physical environment within the nest, control of male and female production and sex ratio, caste differences and caste determination, division of labour, colony multiplication, orientation to the nest and food sources, foraging behaviour and communication and colony defence.

I found the chapters on the social



Stephen Dalton

Social organisation of bees

significance of the nest and its contents and on the handling and transfer of materials within the nest to be the most absorbing because from these two chapters one obtains a vivid evolutionary picture of how a series of relatively simple stimuli, and the flow of food and pheromones between the members of a colony, can result in the co-ordinated activities of many thousands of individuals. These subjects have recently undergone intensive research and it is only by a complete and proper understanding of them that we will be able to discover the reasons for population fluctuations and changes in foraging intensity. Such an understanding will, incidentally, help us to use the honeybee more effectively both as a producer of honey and as a pollinator of crops. For example, Professor Michener's initial discovery that the activity of each halictine bee diminishes with an increase in the size of the colony concerned has been extended to other social bees, including the honeybee, for which it has important practical applications. Readers will, however, be fascinated equally by the chapters on caste determination—a subject that is currently attracting much attention—and on the dance language of bees, the interpretation of which has recently been the subject of a controversy that is summarised skilfully and objectively.

The last chapter in Part II, which is devoted exclusively to the origin and evolution of social behaviour, is in many ways the hub of the book. There, Professor Michener discusses the prerequisites necessary for a species to evolve a social behaviour and the

characteristics associated with different levels of social organisation. He also considers how selection could operate to establish various social attributes paying particular attention to kin selection and the role of altruism.

Part III entitled "Natural History" is an account of the locations, architecture and contents of nests, and a discussion of the general biology, life history, behaviour and social status of each of the main groups of social bees, including the semi-social and eusocial halictine bees, euglossine bees, allodopine bees, bumblebees and the stingless and true honeybees. Professor Michener recommends that readers seeking information on a particular group of social bees should read the relevant section of Part III before checking the index for material in Part I and II. Because of the efficient index this system works well in practice, but Part III could, in effect, have preceded Part II. The book is illustrated liberally; the diagrammatic sketches of nest structure and architecture are especially worthy of attention. There is a glossary, and a useful appendix aimed at relating the up-to-date nomenclature used in the book to any older nomenclature the reader may encounter. The book is documented throughout: half of the 700 or so references were published during the past decade and over 80% during the past two decades, reflecting the current interest and progress in the subject.

The entomologist who studies social insects may well find that other insects are somewhat dull by comparison. Readers of Professor Michener's book will appreciate why. **John B. Free**

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Viruses

Comprehensive Virology. Edited by Heinz Fraenkel-Conrat and Robert R. Wagner. Pp. x+191. (Plenum Press: New York and London, 1974.) \$15.00.

VOLUME 1 of this series is unquestionably comprehensive. The *Descriptive Catalogue of Viruses* lists nearly 500 animal viruses, from "Acado" to "Zika", some 350 plant viruses from "Amapari virus (renavirus)" to three viruses of protists from "A" to "ZS", (both bacteriophages of *Escherichia coli*). The descriptions range from purely taxonomic designations — "Amapari virus (arenavirus)" to three pages of detail on tobacco mosaic virus. There are a few errors, a few eccentricities, (an extra 'i' in -viridae throughout), a few omissions (such as the ephemeral fever virus), and perhaps a few mycoplasmas still masquerading as plant viruses. This is, nevertheless, a unique compilation. **J. S. Porterfield**

Choosing and chancing

Our Future Inheritance: Choice or Chance? A Study by A British Association Working Party. Pp. ix+141+4 plates. (Oxford University: London, October 1974.) £4.00 boards; £1.25 paper.

It has become axiomatic that science, the acquisition of knowledge, should be expected to increase the health, wealth and happiness of mankind. That would seem to apply especially to the results of studies on the various complex processes concerning reproduction, whether they be directed at stimulating fertility or curbing prolificacy.

The British Association and its panel of experts are to be congratulated on this publication. The book, though recognising the benefits accruing from advances in biomedical techniques in the field of reproduction and genetics, urges, quite rightly, that a close watch be kept on possible dangers that could arise from their application to disorders in man.

The terms AID, fertility drug, organ-transplant, test-tube baby, and genetic engineering have all become quite commonplace as a result of popular reporting in the media. The methods and results of the scientific procedures associated with these terms are presented accurately. More important is the awareness that is created of the grave consequences that could follow man's deliberate manipulation and possible interference with his own genes, gametes or gonads. The germ cells, which, strictly speaking, are not vital to the individual are well known for their greater sensitivity to environ-

mental and other changes than are somatic cells.

It is intended that the book should be widely read and it deserves to reach all classes of professional and lay people so that the significance of advances in biological sciences, and their application to human medicine, can be fully realised.

The manner of presentation of a scientific subject in a form acceptable to the lay public is problematical but the authors/editors have overcome skilfully a very difficult situation. I get the impression that the book has been presented in such a way that if members of the public who are scientifically uninformed wish to read and understand it they would obtain all that was needed from the first chapter ('Bio-medical advances: a mixed blessing?') and the last chapter ('Social concern and biological advances'). It is regrettable that those two chapters cannot be made more freely available for all to read and enjoy. The intervening chapters contain details about various technical advances and will require a fair understanding of the relevant subjects if they are to have any lasting effect and impression.

The case-bound volume is well produced and contains a useful index.

I. W. Rowlands

Exercise and sport

Science and Medicine of Exercise and Sport. Edited by Warren R. Johnson and E. R. Buskirk. Pp. 486. (Harper and Row: New York and London, 1973.) £10.00.

THIS volume is a collection of 33 chapters compiled by 40 different authors, linked by a common interest in the physiological and medical aspects of sport and exercise. Each contribution is a short review article; the subjects dealt with range from basic biochemical features of contracting muscle to the effects of exercise on the mental state of psychiatric patients. At the end of each chapter is a list of cited references. There is, however, no general index for the whole book.

I found it difficult to determine for whom the text was intended. The general non-scientific reader would find it incomprehensible. A scientist with no specialised knowledge of the subject would find the format difficult reading: it is fragmented, it lacks a theme, and it is therefore indigestible. Perhaps it would be of interest and use to a student writing a dissertation on the scientific and medical aspects of exercise and sport. Its place would therefore seem to be more appropriate to the shelves of a reference library than to a private bookcase.

R. Y. Calne

Rhythm book never swings

Biological Clocks in Marine Organisms: The Control of Physiological and Behavioural Tidal Rhythms. By J. D. Palmer. Pp. x+173. (Wiley: New York and London, June 1974.) £6.85.

In this book the author attempts to bring the problems and fascinations of the study of biological clocks in marine organisms before a wide audience. The book is, in fact, an expansion of the author's recent review in the journal *Biological Reviews* and is the first of its kind.

It is about the way in which various activities of a whole range of marine animals are instigated and controlled by some facet of the rhythm of the tides. An essential aim of any animal is to maximise the efficiency of, for example, its feeding so that it takes the greatest advantage of food when available. Thus, an intertidal animal which is quiescent when the tide is out undergoes changes when the tide is due to cover it so that it is absolutely ready to feed when water covers it. This preparatory behaviour persists for a period when the animal is removed to a laboratory, demonstrating its innate character. The description of a variety of phenomena of this kind, and the mechanisms behind them, form the substance of this book.

To be completely successful in attracting a wide audience requires subject matter of considerable fascination, a style of writing which is both easily comprehensible and stimulating, and illustrations of high quality. In order to attract future researchers the gaps in present knowledge require emphasis, as do promising lines of research. Unfortunately, although some of these ideals are approached quite closely in some respects the book seems to fall short of its target.

The book is quite comprehensive and incorporates almost all of the significant literature on the subject. It is very well illustrated and the style is generally clear once technical terms are mastered. A very good feature is the summary at the end of each chapter, and the bibliography is of value to the non-specialist.

In spite of that and the obvious expertise of the author, however, I was not greatly stimulated by his treatment of the available material, which seemed rather mundane. I feel that although the general reader is likely to be attracted by the subject he will not be altogether attracted by its presentation; and whether any potential researcher would be fired with enthusiasm is debatable. Dr Palmer does point out the gaps in current knowledge but he never makes one feel that life would



"Circle Limit IV (Heaven and Hell)", a woodcut by Maurits C. Escher. Devils and angels alternate repeatedly and compete for attention. From *Image, Object, and Illusion*. (Readings from *Scientific American*.) Introductions by Richard Held. Pp. 137+155 illustrations. (Freeman: San Francisco and London, 1974.) Cloth, \$8.00; paper, \$4.50.

be incomplete without further knowledge of this or that phenomenon.

The book is undoubtedly a very useful contribution to literature and should be in every biology department's library but its contribution lies in its value as a storehouse of information rather than as a source of inspiration. Its price will probably mean that few other than libraries will buy it.

R. H. Emson

Pain

Recent Advances on Pain. Edited by John J. Bonica, P. Procacci, and C. A. Pagni. Pp. xv+373. (Charles C. Thomas: Springfield, 1974.) \$19.75.

THIS excellent book contains the proceedings of a symposium on the pathophysiology and clinical aspects of pain held in Florence in April 1972. The contributors were eminent men, representing various specialties, from Italy, Switzerland, North America and the United Kingdom.

The main message of the symposium is reflected by its broad coverage—namely, that for both the study of pain and its clinical management a multidisciplinary approach is needed. The subject matter included the neurophysiology of pain, endogenous pain-producing substances, analgesic blocks

and surgery for the relief of pain, pain threshold measurements in man, and operant conditioning for chronic pain. In addition, there is a paper by Professor Bonica on the organisation and function of a pain clinic.

This list, although giving some idea of the scope of this book, does not do justice to the vast amount of information it contains. For those interested in migraine there is a fascinating contribution by Professor Sicuteri, and there are some thoughts on the roles of acupuncture, radiostimulators and inhibitory mechanisms in the relief of pain.

In order that the fruits of many years of research undertaken by the contributors be disseminated as widely as possible this book should be read not only by those whose research is directed towards the study of pain but also by those who are in any way connected with the relief of suffering.

The editing of a book of this kind must be a vast undertaking; none the less, it is a pity that there are so many errors. Apart from spelling mistakes and what seem to be mistakes arising from misread handwriting it is strange that there are 65 references at the end of Chapter VIII when only nine are mentioned in the text.

Diana R. Haslam

announcements

Awards

The Royal Society medals for 1974 have been awarded to the following:

The Copley Medal to Sir William Hodge.

The Rumford Medal to Sir Alan Cottrell.

A Royal Medal to Sir Fred Hoyle.

A Royal Medal to S. Brenner.

A Royal Medal to Sir George Edwards.

The Davy Medal to J. Baddiley.

The Darwin Medal to P. M. Sheppard.

The Hughes Medal to P. H. Fowler.

The Mullard Medal to F. B. Mercer.

The Esso Medal to K. A. Bray.

International meetings

February 11–14, **Spectrum Utilisation in Radio Communication**, London (Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2R 0BL).

February 12–14, **Solid State Circuits**, Philadelphia (The Institute of Electrical and Electronics Engineers Technical Activities Board, 345 East 47th Street, New York, NY 10017, USA).

February 21, **Particle Detectors and Dosimeters**, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX).

March 12–14, **Particle Accelerator Conference**, Washington (The Institute of Electrical and Electronics Engineers Technical Activities Board, 345 East 47th Street, New York, New York 10017, USA).

March 17–20, **Conference on Surfaces**, Warwick (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX).

March 17–25, **Oceanology International 75**, Brighton (BPS Exhibitions Ltd., 4 Seaford Court, 220–222 Great Portland Street, London W1N 5HH).

March 23–29, **5th International Congress on Lymphology**, Buenos Aires/Rio de Janeiro (Professor C. R. Mayall, CP 1822 (2C-00) Rio de Janeiro-GB, Brazil or Dr C. M. Grandval, Austria 2636, Buenos Aires, Argentina).

March 24–25, **Zmuda Memorial Conference on Geomagnetic Field Models**, Colorado Springs (American Geophysical Union, 1707 L Street, NW, Washington, DC 20036, USA).

March 24–26, **Europhysics Conference on Nuclear Interactions at Medium and Low Energies**, Harwell (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

March 31–April 4, **2nd International Conference on Clustering Phenomena**, College Park, USA (Dr Harry D. Holmgren, Division de Physique Nucleaire, Institut de Physique Nucleaire, BP no. 1 F-91, Orsay, France).

Reports and publications

Great Britain

Department of Industry. Technology and the Environment—Reports from Scientific Counsellors, No. 7. Pp. 56. (London: Department of Industry, 1974.) [1510]

Ministry of Agriculture, Fisheries and Food. Report of the Director of Marine Fishery Research, January 1972/March 1973. Pp. 72. (Lowestoft, Suffolk: Fisheries Laboratory, 1974.) [2110]

The Panelled Ceiling of the Natural History Museum. (Botany Leaflet No. 1.) Pp. 12. (London: British Museum (Natural History), 1974.) 10p. [2310]

Venerable Diseases. (OHE Briefing, No. 1, October 1974.) Pp. 6. (London: Office of Health Economics, 1974.) [2310]

Forestry Commission. Fifty-Third Annual Report and Accounts, 1972/73. Pp. 112 + 8 plates, 99p. net. Bulletin 47: Work Study in Forestry. Edited by W. O. Wittering. Pp. vii + 190 + 14 plates. £1 net. Forest Record No. 87: Hydraulic Grapple Cranes for Forest Use. By F. B. W. Platt. Pp. 37. 30p. net. (London: HMSO, 1974.) [2510]

Republic of Ireland. Annual Report of the National Drugs Advisory Board, 1973. Pp. 39. (Dublin: National Drugs Advisory Board, 1974.) [2510]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 277, No. 1267: Large Amplitude Waves in Bounded Media. II. The Deformation in an Impulsively Loaded Slab: The First Reflexion. III. The Deformation of an Impulsively Loaded Slab: The Second Reflexion. By J. Y. Kazakia and E. Varley. Pp. 191–150. (London: The Royal Society, 1974.) [2510]

Department of Education and Science. Welsh Office. Scottish Education Department. Department of Education for Northern Ireland. University Grants Committee. Education Statistics for the United Kingdom, 1972. Pp. xxvii + 86. (London: HMSO, 1974.) £1.90 net. [2810]

Engineering Optimization, Vol. 1, No. 1, 1974. Edited by Andrew B. Templeman. Pp. 1–70. Subscription Rates: Great Britain: Individuals who warrant that the journal is for their own personal use and who order direct from the publisher, £6; Libraries, Institutions and others £20. USA/Elsewhere, Individuals \$15.50/£6.50; Libraries etc. \$52/£22.50. (London and New York: Gordon and Breach Science Publishers, Ltd., 1974.) [2910]

Electrocomponent Science and Technology, Vol. 1, No. 1, 1974. Edited by D. S. Campbell. Pp. 1–86. Subscription Rates (per volume of four issues). Great Britain: Individuals who warrant that the journal is for their own personal use and who order direct from the publisher, £7; Libraries, Institutions and others £24. USA/Elsewhere: Individuals \$21 (£9); Libraries etc. \$60 (£26). (London and New York: Gordon and Breach, 1974.) [111]

Other Countries

New Zealand: Department of Health. Environmental Radioactivity Annual Report, 1973. Pp. 37. (Christchurch, NZ: National Radiation Laboratory, 1974.) [1010]

Sveriges Geologiska Undersökning. Arsberättelse för År 1973. Pp. 47. Avhandlingar och Uppsatser. Ser. C. No. 705: Reference Localities for Palaeontology and Geology in the Silurian of Gotland. By Sven Laufeld. Pp. 172. Ser. Ag., No. 2: Beskrivning Till Hydrogeologiska Kartblad Orebö No. (Description of the Hydrogeological Map Orebö No.) By A. Möller, B. Jarnefors, P. Engqvist and C. F. Mullern. Pp. 47. (Stockholm: Sveriges Geologiska Undersökning, 1974. Distribution: Svenska Reproduktions AB, Fack, 162 10 Vällingby 1.) [1010]

Bulletin of the Fisheries Research Board of Canada, No. 188: Aquaculture in Canada—The Practice and the Promise. By H. R. MacCrimmon, J. E. Stewart and J. R. Brett. Pp. x + 84. (Ottawa: Information Canada, 1974.) \$4.50. [1110]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 230: Triassic Rocks of the Southern Canadian Rocky Mountains. By D. W. Gibson. Pp. 65 (8 plates). (Ottawa: Information Canada, 1974.) \$4. [1110]

Science Council of Canada. Issues in Canadian Science Policy, No. 1. Pp. ii + 31. (Ottawa: Information Canada, 1974.) \$1. [1110]

Fundación Universidad de Bogotá Jorge Tadeo Lozano—Facultad de Ciencias del Mar. Museo del Mar. Boletín No. 6: Peces Colectados en el Caribe Colombiano por la Universidad de Miami. Por Francisco J. Palacio. Pp. 137. (Bogotá, Colombia: Museo del Mar, 1974.) [1110]

World Health Organization. Technical Report Series, No. 554: Health Aspects of Environmental Pollution Control—Planning and Implementation of National Programmes: Report of a WHO Expert Committee. Pp. 57. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 6. [1510]

Smithsonian Contributions to Botany, No. 16: Morden-Smithsonian Expedition to Dominica—The Lichens (Thelotremaaceae). By Mason E. Hale, jun. Pp. iii + 46. \$1.05. Smithsonian Contributions to Zoology, No. 172: The So-called Cheirodontin Fishes

of Central America with Descriptions of Two New Species (Pisces: Characidae). By William L. Fink and Stanley H. Weitzman. Pp. iii + 46. \$1.05. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [1710]

World Health Organization. Trace Elements in Relation to Cardiovascular Disease, (Status of the Joint WHO/IAEA Research Programme). Edited by R. Masironi. Pp. 45. Sw. fr. 7. Technical Report Series, No. 550: Fish and Shellfish Hygiene—Report of a WHO Expert Committee convened in co-operation with FAO. Pp. 62. Sw. fr. 6. (Geneva: WHO; London: HMSO, 1974.) [2110]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 232: Conodonts of the Waterways Formation (Upper Devonian) of Northeastern and Central Alberta. By T. T. Uyeno. Pp. 93 (8 plates). (Ottawa: Information Canada, 1974.) \$4. [2110]

United States: Department of the Interior: Geological Survey. Water-Supply Paper 2001-D: Appraisal of Operating Efficiency of Recharge Basins on Long Island, New York, in 1969. By D. A. Aronson and G. E. Seaburn. Pp. iv + 22 + plate 1. \$1.45. Professional Paper 655-H: Quantitative and Historical Evidence of Vegetation Changes Along the Upper Gila River, Arizona. By Raymond M. Turner. Pp. v + 20 + plate 1. \$2. Professional Paper 811: Geology of Salars in Northern Chile. By George E. Stoertz and George E. Erickson. Pp. iv + 65. \$1.65. Professional Paper 831-B: General Geology of the Harold D. Roberts Tunnel, Colorado. By Charles S. Robinson, Lawrence A. Warner and Ernest E. Wahlstrom. Pp. iv + 48 + plate 1. \$2.25. (Washington, DC: Government Printing Office, 1974.) [2210]

The Effects of Developments in the Biological and Chemical Sciences on CW Disarmament Negotiations. Pp. 54. (Stockholm: Stockholm International Peace Research Institute, 1974.) [2310]

Smithsonian Contributions to the Earth Sciences, No. 13: Distinctive Properties of Turbiditic and Hemipelagic Mud Layers in the Algero-Balearic Basin, Western Mediterranean Sea. By N. A. Rucpe and D. J. Stanley. Pp. iii + 40. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 95 cents. [2310]

Radioactive Effluents from Nuclear Power Stations in the Community—Discharge Data, Radiological Aspects. Pp. 19 + 13 tables. (Luxembourg: Commission of the European Communities, Directorate-General for Social Affairs, 1974.) [2310]

Annual Summary of Information on Natural Disasters: Earthquakes, Tsunamis, Volcanic Eruptions, Landslides, Avalanches No. 7, 1972. Pp. 106. (Paris: The Unesco Press; London: HMSO, 1974.) [2510]

Records of the Australian Museum, Vol. 29, No. 9: *Cymodetta gambosa*, a New Sphaeromata Isopod (Crustacea) from Australia, with Notes on Its Mating Behaviour. By Thomas E. Bowman and Helmut Kuhne. Pp. 235–244 + plate 8. (Sydney: The Australian Museum, 1974.) 50c. [2520]

Suomalaisen Tiedeakatemian Toimituksia. Annales Academiae Scientiarum Fennicae. Ser. B, Tom 184: Neural Coding in the Temperature Sense: Human Reactions to Temperature Changes as Compared with Activity in Single Peripheral Cold Fibres in the Cat. By T. Jarvilehto. Pp. 71. (Helsinki: Suomalainen Tiedeakatemia, 1973.) [2810]

Science Research Council of Canada. Report No. 22: Science for Health Services. Pp. 140. (Ottawa: Information Canada, 1974.) \$2. [2810]

Smithsonian Contributions to Botany, No. 14: Commercial Timbers of West Africa. By Edward S. Ayensu and Albert Bentum. Pp. 69. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.65. [2810]

Australian Journal of Zoology. Supplementary Series No. 28: A Revision of the Australian and New Guinea Predatory Ceratopogonidae (Diptera: Nematocera) of the Tribes Heteromyiini and Sphaeromyiini. By Margaret L. Debenham. Pp. 92. No. 29: Comments on the Taxonomy of Australian Austroperlidae and Gripopterygidae (Plecoptera). Observations on the Adults and Eggs of Australian Plecoptera. By H. B. N. Hynes. Pp. 52. (East Melbourne: CSIRO, 372 Albert Street, 1974.) [2810]

World Health Organization. Public Health Papers No. 56: The Training and Utilisation of Fieldshers in the USSR. (A Review prepared by the Ministry of Health of the USSR for the WHO.) Pp. 52. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 5. [2910]

Induced Mutations for Disease Resistance in Crop Plants. (Proceedings of a Research Coordination Meeting organised by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture and the Swedish International Development Authority (SIDA), and held in Novi Sad, June, 4–8 1973. Panel Proceedings Series.) Pp. 193. (Vienna: IAEA; London: HMSO, 1974.) 182 schillings; £4; \$10. [2910]

World Health Organization. Technical Report Series, No. 555: The Use of Mercury and Alternative Compounds as Seed Dressings—Report of a Joint FAO/WHO Meeting. Pp. 29. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 5. [2910]

Institut Royal Météorologique de Belgique. Publications Série B. No. 76: Persistence de la Direction Moyenne Horaire du Vent dans la Couche Limite Planétaire. Par L. M. Malet et H. Bultynck. Pp. 38. (Bruxelles: Institut Royal Météorologique de Belgique, 1974.) [3010]

Studia Forestalia Suecica. No. 116: A Study on the Temperature Response of Pollen Mother Cells in Norway Spruce. By Alena Jonsson. Pp. 32. No. 117: Microscopic Studies on the Degradation of Cellophane and Various Cellulosic Fibres by Wood-Attacking Microfungi. By Thomas Nilsson. Pp. 32. No. 118: Seed Development After Self-Pollination and Cross-Pollination of Scots Pine, *Pinus sylvestris* L. By Christina Plym Forsell. Pp. 37. (Stockholm: Skogshögskolan, Royal College of Forestry, 1974.) [3110]